factor 1-40, human; growth hormone releasing factor 1-40, amide, human; growth hormone releasing factor 30-44, amide, human; growth hormone releasing factor, mouse; growth hormone releasing factor, ovine; growth hormone releasing factor, rat; biotinyl- growth hormone releasing factor, rat; GHRP-6 ([His^{1}, Lys^{6}]-GHRP); hexarelin (growth hormone releasing hexapeptide); and [$D\text{-}Lys^{3}$]-GHRP-6.

GTP-binding protein fragment peptides including, but not limited to, [Arg^{8}]-GTP-binding protein fragment, Gs alpha; GTP-binding protein fragment, G beta; GTP-binding protein fragment, GAlpha; GTP-binding protein fragment, Go Alpha; GTP-binding protein fragment, Gs Alpha; and GTP-binding protein fragment, G Alpha i2.

Guanylin peptides including, but not limited to, guanylin, human; guanylin, rat; and uroguanylin.

Inhibin peptides including, but not limited to, inhibin, bovine; inhibin, alpha-subunit 1-32, human; [Tyr^{0}]-inhibin, alpha-subunit 1-32, human; seminal plasma inhibin-like peptide, human; [Tyr^{0}]-seminal plasma inhibin-like peptide, human; inhibin, alpha-subunit 1-32, porcine; and [Tyr^{0}]-inhibin, alpha-subunit 1-32, porcine.

Insulin peptides including, but not limited to, insulin, human; insulin, porcine; IGF-I, human; insulin-like growth factor II (69-84); pro-insulin-like growth factor II (68-102), human; pro-insulin-like growth factor II (105-128), human; [Asp^{B28}]-insulin, human; [Lys^{B28}]-insulin, human; [Leu^{B28}]-insulin, human; [Val^{B28}]-insulin, human; [Ala^{B28}]-insulin, human; [Asp^{B28}, Pro^{B29}]-insulin, human; [Lys^{B28}, Pro^{B29}]-insulin, human; [Leu^{B28}, Pro^{B29}]-insulin, human; [Val^{B28}, Pro^{B29}]-insulin, human; and [Ala^{B28}, Pro^{B29}]-insulin, human, B22-B30 insulin, human; B23-B30 insulin, human; B25-B30 insulin, human; B26-B30 insulin, human; B27-B30 insulin, human; B29-B30 insulin, human; the A chain of human insulin, and the B chain of human insulin.

Interleukin peptides including, but not limited to, interleukin-1 beta 165-181, rat; and interleukin-8 (IL-8, CINC/gro), rat.

Laminin peptides including, but not limited to, laminin; alpha1 (I)-CB3 435-438, rat; and laminin binding inhibitor.

Leptin peptides including, but not limited to, leptin 93-105, human; leptin 22-56, rat; Tyr-leptin 26-39, human; and leptin 116-130, amide, mouse.

Leucokinin peptides including, but not limited to, leucomyosuppressin (LMS); leucopyrokinin (LPK); leucokinin I; leucokinin II; leucokinin III; leucokinin IV; leucokinin VI; leucokinin VII; and leucokinin VIII.

Luteinizing hormone-releasing hormone peptides including, but not limited to, antide; Gn-RH II, chicken; luteinizing hormone-releasing hormone (LH-RH) (GnRH); biotinyl-LH-RH; cetrorelix (D-20761); [D-Ala^{6}]-LH-RH; [Gln^{8}]-LH-RH (Chicken LH-RH); [DLeu^{6}, Val^{7}] LH-RH 1-9, ethyl amide; [D-Lys^{6}]-LH-RH; [D-Phe^{2}, Pro^{3}, D-Phe^{6}]-LH-RH; [DPhe^{2}, DAla^{6}] LH-RH; [Des-Gly^{10}]-LH-RH, ethyl amide; [D-Ala^{6}, Des-Gly^{10}]-LH-RH, ethyl amide; [DTrp^{6}]-LH-RH, ethyl amide; [D-Trp^{6}, Des-Gly^{10}]-LH-RH, ethyl amide (Deslorelin); [DSer(But)^{6}, Des-Gly^{10}]-LH-RH, ethyl amide; ethyl amide; leuprolide; LH-RH 4-10; LH-RH 7-10; LH-RH, free acid; LH-RH, lamprey; LH-RH, salmon; [Lys^{8}]-LH-RH; [Trp^{7},Leu^{8}] LH-RH, free acid; and [(t-Bu)DSer^{6}, (Aza)Gly^{10}]-LH-RH.

Mastoparan peptides including, but not limited to, mastoparan; mas7; mas8; mas17; and mastoparan X.

Mast cell degranulating peptides including, but not limited to, mast cell degranulating peptide HR-1; and mast cell degranulating peptide HR-2.

Melanocyte stimulating hormone (MSH) peptides including, but not limited to, [Ac-Cys^{4},DPhe^{7},Cys^{10}] alpha-MSH 4-13, amide; alpha-melanocyte stimulating hormone; alpha-MSH, free acid; beta-MSH, porcine; biotinyl-alpha-melanocyte stimulating hormone; biotinyl-[Nle^{4}, D-Phe^{7}] alpha-melanocyte stimulating hormone; [Des -Acetyl]-alpha-MSH; [DPhe^{7}]-alpha-MSH, amide; gamma-1-MSH, amide; [Lys^{0}]-gamma-1-MSH, amide; MSH release inhibiting factor, amide; [Nle^{4}]-alpha-MSH, amide; [Nle^{4}, D-Phe^{7}]-alpha-MSH; N-Acetyl, [Nle^{4},DPhe^{7}] alpha-MSH 4-10, amide; beta-MSH, human; and gamma-MSH.

Morphiceptin peptides including, but not limited to, morphiceptin (beta-casomorphin 1-4 amide); [D-Pro^{4}]-morphiceptin; and [N-MePhe^{3},D-Pro^{4}]-morphiceptin.

Motilin peptides including, but not limited to, motilin, canine; motilin, porcine; biotinyl-motilin, porcine; and [Leu^{13}]-motilin, porcine.

Neuro-peptides including, but not limited to, Ac-Asp-Glu; achatina cardio-excitatory peptide-1 (ACEP-1) (Achatina fulica); adipokinetic hormone (AKH) (Locust); adipokinetic hormone (Heliothis zea and Manduca sexta); alytesin; Tabanus atratus adipokinetic hormone (Taa-AKH); adipokinetic hormone II (Locusta migratoria); adipokinetic hormone II (Schistocera gregaria); adipokinetic hormone III (AKH-3); adipokinetic hormone G (AKH-

G) (Gryllus bimaculatus); allatotropin (AT) (Manduca sexta); allatotropin 6-13 (Manduca sexta); APGW amide (Lymnaea stagnalis); buccalin; cerebellin; [Des-Ser^1]-cerebellin; corazonin (American Cockroach Periplaneta americana); crustacean cardioactive peptide (CCAP); crustacean erythrophore; DF2 (Procambarus clarkii); diazepam-binding inhibitor fragment, human; diazepam binding inhibitor fragment (ODN); eledoisin related peptide; FMRF amide (molluscan cardioexcitatory neuro-peptide); Gly-Pro-Glu (GPE), human; granuliberin R; head activator neuropeptide; [His^7]-corazonin; stick insect hypertrehalosaemic factor II; Tabanus atratus hypotrehalosemic hormone (Taa-HoTH); isoguvacine hydrochloride; bicuculline methiodide; piperidine-4-sulphonic acid; joining peptide of proopiomelanocortin (POMC), bovine; joining peptide, rat; KSAYMRF amide (P. redivivus); kassinin; kinetensin; levitide; litorin; LUQ 81-91 (Aplysia californica); LUQ 83-91 (Aplysia californica); myoactive peptide I (Periplanetin CC-1) (Neuro-hormone D); myoactive peptide II (Periplanetin CC-2); myomodulin; neuron specific peptide; neuron specific enolase 404-443, rat; neuropeptide FF; neuropeptide K, porcine; NEI (prepro-MCH 131-143) neuropeptide, rat; NGE (prepro-MCH 110-128) neuropeptide, rat; NFI (Procambarus clarkii); PBAN-1 (Bombyx mori); Hez-PBAN (Heliothis zea); SCPB (cardioactive peptide from aplysia); secretoneurin, rat; uperolein; urechistachykinin I; urechistachykinin II; xenopsin-related peptide I; xenopsin-related peptide II; pedal peptide (Pep), aplysia; peptide F1, lobster; phyllomedusin; polistes mastoparan; proctolin; ranatensin; Ro I (Lubber Grasshopper, Romalea microptera); Ro II (Lubber Grasshopper, Romalea microptera); SALMF amide 1 (S1); SALMF amide 2 (S2); and SCPA.

Neuropeptide Y (NPY) peptides including, but not limited to, [Leu^{31},Pro^{34}]-neuropeptide Y, human; neuropeptide F (Moniezia expansa); B1BP3226 NPY antagonist; Bis (31/31') {[Cys^{31}, Trp^{32}, Nva^{34}] NPY 31-36}; neuropeptide Y, human, rat; neuropeptide Y 1-24 amide, human; biotinyl-neuropeptide Y; [D-Tyr^{27,36}, D-Thr^{32}]-NPY 27-36; Des 10-17 (cyclo 7-21) [Cys^{7,21}, Pro^{34}]-NPY; C2-NPY; [Leu^{31}, Pro^{34}] neuropeptide Y, human; neuropeptide Y, free acid, human; neuropeptide Y, free acid, porcine; prepro NPY 68-97, human; N-acetyl-[Leu^{28}, Leu^{31}] NPY 24-36; neuropeptide Y, porcine; [D-Trp^{32}]-neuropeptide Y, porcine; [D-Trp^{32}] NPY 1-36, human; [Leu^{17},DTrp^{32}] neuropeptide Y, human; [Leu^{31}, Pro^{34}]-NPY, porcine; NPY 2-36, porcine; NPY 3-36, human; NPY 3-36, porcine; NPY 13-36, human; NPY 13-36, porcine; NPY 16-36, porcine; NPY 18-36, porcine; NPY 20-36;

NPY 22-36; NPY 26-36; [Pro^{34}]-NPY 1-36, human; [Pro^{34}]-neuropeptide Y, porcine; PYX-1; PYX-2; T4-[NPY(33-36)]4; and Tyr(OMe)^{21}]-neuropeptide Y, human.

Neurotropic factor peptides including, but not limited to, glial derived neurotropic factor (GDNF); brain derived neurotropic factor (BDNF); and ciliary neurotropic factor (CNTF).

Orexin peptides including, but not limited to, orexin A; orexin B, human; orexin B, rat, mouse.

Opioid peptides including, but not limited to, alpha-casein fragment 90-95; BAM-18P; casomokinin L; casoxin D; crystalline; DALDA; dermenkephalin (deltorphin) (Phylomedusa sauvagei); [D-Ala^2]-deltorphin I; [D-Ala^2]-deltorphin II; endomorphin-1; endomorphin-2; kyotorphin; [DArg^2]-kyotorphin; morphin tolerance peptide; morphine modulating peptide, C-terminal fragment; morphine modulating neuropeptide (A-18-F-NH2); nociceptin [orphanin FQ] (ORL1 agonist); TIPP; Tyr-MIF-1; Tyr-W-MIF-1; valorphin; LW-hemorphin-6, human; Leu-valorphin-Arg; and Z-Pro-D-Leu.

Oxytocin peptides including, but not limited to, [Asu^6]-oxytocin; oxytocin; biotinyl-oxytocin; [Thr^4, Gly^7]-oxytocin; and tocinoic acid ([Ile^3]-pressinoic acid).

PACAP (pituitary adenylating cyclase activating peptide) peptides including, but not limited to, PACAP 1-27, human, ovine, rat; PACAP (1-27)-Gly-Lys-Arg-NH2, human; [Des-Gln^{16}]-PACAP 6-27, human, ovine, rat; PACAP38, frog; PACAP27-NH2, human, ovine, rat; biotinyl-PACAP27-NH2, human, ovine, rat; PACAP 6-27, human, ovine, rat; PACAP38, human, ovine, rat; biotinyl-PACAP38, human, ovine, rat; PACAP 6-38, human, ovine, rat; PACAP27-NH2, human, ovine, rat; biotinyl-PACAP27-NH2, human, ovine, rat; PACAP 6-27, human, ovine, rat; PACAP38, human, ovine, rat; biotinyl-PACAP38, human, ovine, rat; PACAP 6-38, human, ovine, rat; PACAP38 16-38, human, ovine, rat; PACAP38 31-38, human, ovine, rat; PACAP38 31-38, human, ovine, rat; PACAP-related peptide (PRP), human; and PACAP-related peptide (PRP), rat.

Pancreastatin peptides including, but not limited to, chromostatin, bovine; pancreastatin (hPST-52) (chromogranin A 250-301, amide); pancreastatin 24-52 (hPST-29), human; chromogranin A 286-301, amide, human; pancreastatin, porcine; biotinyl-pancreastatin, porcine; [Nle^8]-pancreastatin, porcine; [Tyr^0,Nle^8]-pancreastatin, porcine; [Tyr^0]-pancreastatin, porcine; parastatin 1-19 (chromogranin A 347--365), porcine; pancreastatin (chromogranin A 264-314-amide, rat; biotinyl-pancreastatin (biotinyl-

chromogranin A 264-314-amide; [Tyr^0]-pancreastatin, rat; pancreastatin 26-51, rat; and pancreastatin 33-49, porcine.

Pancreatic polypeptides including, but not limited to, pancreatic polypeptide, avian; pancreatic polypeptide, human; C-fragment pancreatic polypeptide acid, human; C-fragment pancreatic polypeptide amide, human; pancreatic polypeptide (Rana temporaria); pancreatic polypeptide, rat; and pancreatic polypeptide, salmon.

Parathyroid hormone peptides including, but not limited to, [Asp^{76}]-parathyroid hormone 39-84, human; [Asp^{76}]-parathyroid hormone 53-84, human; [Asn^{76}]-parathyroid hormone 1-84, hormone; [Asn^{76}]-parathyroid hormone 64-84, human; [Asn^8, Leu^{18}]-parathyroid hormone 1-34, human; [$Cys^{5,28}$]-parathyroid hormone 1-34, human; hypercalcemia malignancy factor 1-40; [Leu^{18}]-parathyroid hormone 1-34, human; [$Lys(biotinyl)^{13}$, $Nle^{8,18}$, Tyr^{34}]-parathyroid hormone 1-34 amide; [$Nle^{8,18}$, Tyr^{34}]-parathyroid hormone 1-34 amide; [$Nle^{8,18}$, Tyr^{34}]-parathyroid hormone 3-34 amide, bovine; [$Nle^{8,18}$, Tyr^{34}]-parathyroid hormone 1-34, human; [$Nle^{8,18}$, Tyr^{34}]-parathyroid hormone 1-34 amide, human; [$Nle^{8,18}$, Tyr^{34}]-parathyroid hormone 3-34 amide, human; [$Nle^{8,18}$, Tyr^{34}]- parathyroid hormone 7-34 amide, bovine; [$Nle^{8,21}$, Tyr^{34}]-parathyroid hormone 1-34 amide, rat; parathyroid hormone 44-68, human; parathyroid hormone 1-34, bovine; parathyroid hormone 3-34, bovine; parathyroid hormone 1-31 amide, human; parathyroid hormone 1-34, human; parathyroid hormone 13-34, human; parathyroid hormone 1-34, rat; parathyroid hormone 1-38, human; parathyroid hormone 1-44, human; parathyroid hormone 28-48, human; parathyroid hormone 39-68, human; parathyroid hormone 39-84, human; parathyroid hormone 53-84, human; parathyroid hormone 69-84, human; parathyroid hormone 70-84, human; [Pro^{34}]-peptide YY (PYY), human; [Tyr^0]-hypercalcemia malignancy factor 1-40; [Tyr^0]-parathyroid hormone 1-44, human; [Tyr^0]-parathyroid hormone 1-34, human; [Tyr^1]-parathyroid hormone 1-34, human; [Tyr^{27}]-parathyroid hormone 27-48, human; [Tyr^{34}]-parathyroid hormone 7-34 amide, bovine; [Tyr^{43}]-parathyroid hormone 43-68, human; [Tyr^{52}, Asn^{76}]-parathyroid hormone 52-84, human; and [Tyr^{63}]-parathyroid hormone 63-84, human.

Parathyroid hormone (PTH)-related peptides including, but not limited to, PTHrP ([Tyr^{36}]-PTHrP 1-36 amide), chicken; hHCF-(1-34)-NH2 (humoral hypercalcemic factor), human; PTH-related protein 1-34, human; biotinyl-PTH-related protein 1-34, human; [Tyr^0]-PTH-related protein 1-34, human; [Tyr^{34}]-PTH-related protein 1-34 amide, human; PTH-related protein 1-37, human; PTH-related protein 7-34 amide, human; PTH-related protein

38-64 amide, human; PTH-related protein 67-86 amide, human; PTH-related protein 107-111, human, rat, mouse; PTH-related protein 107-111 free acid; PTH-related protein 107-138, human; and PTH-related protein 109-111, human.

Peptide T peptides including, but not limited to, peptide T; [D-Ala^1]-peptide T; and [D-Ala^1]-peptide T amide.

Prolactin-releasing peptides including, but not limited to, prolactin-releasing peptide 31, human; prolactin-releasing peptide 20, human; prolactin-releasing peptide 31, rat; prolactin-releasing peptide 20, rat; prolactin-releasing peptide 31, bovine; and prolactin-releasing peptide 20, bovine.

Peptide YY (PYY) peptides including, but not limited to, PYY, human; PYY 3-36, human; biotinyl-PYY, human; PYY, porcine, rat; and [Leu^{31}, Pro^{34}]-PYY, human.

Renin substrate peptides including, but not limited to, acetyl, angiotensinogen 1-14, human; angiotensinogen 1-14, porcine; renin substrate tetradecapeptide, rat; [Cys^8]-renin substrate tetradecapeptide, rat; [Leu^8]-renin substrate tetradecapeptide, rat; and [Val^8]-renin substrate tetradecapeptide, rat.

Secretin peptides including, but not limited to, secretin, canine; secretin, chicken; secretin, human; biotinyl-secretin, human; secretin, porcine; and secretin, rat.

Somatostatin (GIF) peptides including, but not limited to, BIM-23027; biotinyl-somatostatin; biotinylated cortistatin 17, human; cortistatin 14, rat; cortistatin 17, human; [Tyr^0]-cortistatin 17, human; cortistatin 29, rat; [D-Trp^8]-somatostatin; [DTrp^8,DCys^{14}]-somatostatin; [DTrp^8,Tyr^{11}]-somatostatin; [D-Trp^{11}]-somatostatin; NTB (Naltriben); [Nle^8]-somatostatin 1-28; octreotide (SMS 201-995); prosomatostatin 1-32, porcine; [Tyr^0]-somatostatin; [Tyr^1]-somatostatin; [Tyr^1]-somatostatin 28 (1-14); [Tyr^{11}]-somatostatin; [Tyr^0, D-Trp^8]-somatostatin; somatostatin; somatostatin antagonist; somatostatin-25; somatostatin-28; somatostatin 28 (1-12); biotinyl-somatostatin-28; [Tyr^0]-somatostatin-28; [Leu^8, D-Trp^{22}, Tyr^{25}]-somatostatin-28; biotinyl-[Leu^8, D-Trp^{22}, Tyr^{25}]-somatostatin-28; somatostatin-28 (1-14); and somatostatin analog, RC-160.

Substance P peptides including, but not limited to, G protein antagonist-2; Ac-[Arg^6, Sar^9, Met(02)^{11}]-substance P 6-11; [Arg^3]-substance P; Ac-Trp-3,5-bis(trifluoromethyl) benzyl ester; Ac-[Arg^6, Sar^9, Met(O2)^{11}]-substance P 6-11; [D-Ala^4]-substance P 4-11; [Tyr^6, D-Phe^7, D-His^9]-substance P 6-11 (sendide); biotinyl-substance P; biotinyl-NTE[Arg^3]-substance P; [Tyr^8]-substance P; [Sar^9, Met(O2)^{11}]-substance P; [D-Pro^2, D-Trp^{7,9}]-substance

P; [D-Pro^{4}, 0-Trp^{7,9}]-substance P 4-11; substance P 4-11; [DTrp^{2,7,9}]-substance P; [(Dehydro)Pro^{2,4}, Pro^{9}]-substance P; [Dehydro-Pro^{4}]-substance P 4-11; [Glp^{5},(Me)Phe^{8},Sar^{9}]-substance P 5-11; [Glp^{5},Sar^{9}]-substance P 5-11; [Glp^{5}]-substance P 5-11; hepta-substance P (substance P 5-11); hexa-substance P(substance P 6-11); [MePhe^{8},Sar^{9}]-substance P; [Nle^{11}]-substance P; Octa-substance P(substance P 4-11); [pGlu^{1}]-hexa-substance P ([pGlu^{6}]-substance P 6-11); [pGlu^{6}, D-Pro^{9}]-substance P 6-11; [(pNO2)Phe^{7}Nle^{11}]-substance P; penta-substance P (substance P 7-11); [Pro^{9}]-substance P; GR73632, substance P 7-11; [Sar^{4}]-substance P 4-11; [Sar^{9}]-substance P; septide ([pGlu^{6}, Pro^{9}]-substance P 6-11); spantide I; spantide II; substance P; substance P, cod; substance P, trout; substance P antagonist; substance P-Gly-Lys-Arg; substance P 1-4; substance P 1-6; substance P 1-7; substance P 1-9; deca-substance P (substance P 2-11); nona-substance P (substance P 3-11); substance P tetrapeptide (substance P 8-11); substance P tripeptide (substance P 9-11); substance P, free acid; substance P methyl ester; and [Tyr^{8},Nle^{11}] substance P.

Tachykinin peptides including, but not limited to, [Ala^{5}, beta-Ala^{8}] neurokinin A 4-10; eledoisin; locustatachykinin I (Lom-TK-I) (Locusta migratoria); locustatachykinin II (Lom-TK-II) (Locusta migratoria); neurokinin A 4-10; neurokinin A (neuromedin L, substance K); neurokinin A, cod and trout; biotinyl-neurokinin A (biotinyl-neuromedin L, biotinyl-substance K); [Tyr^{0}]-neurokinin A; [Tyr^{6}]-substance K; FR64349; [Lys^{3}, Gly^{8}-(R)-gamma-lactam-Leu^{9}]-neurokinin A 3-10; GR83074; GR87389; GR94800; [Beta-Ala^{8}]-neurokinin A 4-10; [Nle^{10}]-neurokinin A 4-10; [Trp^{7}, beta-Ala^{8}]-neurokinin A 4-10; neurokinin B (neuromedin K); biotinyl-neurokinin B (biotinyl-neuromedin K); [MePhe^{7}]-neurokinin B; [Pro^{7}]-neurokinin B; [Tyr^{0}]-neurokinin B; neuromedin B, porcine; biotinyl-neuromedin B, porcine; neuromedin B-30, porcine; neuromedin B-32, porcine; neuromedin B receptor antagonist; neuromedin C, porcine; neuromedin N, porcine; neuromedin (U-8), porcine; neuromedin (U-25), porcine; neuromedin U, rat; neuropeptide-gamma (gamma-preprotachykinin 72-92); PG-KII; phyllolitorin; [Leu^{8}]-phyllolitorin (Phyllomedusa sauvagei); physalaemin; physalaemin 1-11; scyliorhinin II, amide, dogfish; senktide, selective neurokinin B receptor peptide; [Ser^{2}]-neuromedin C; beta-preprotachykinin 69-91, human; beta-preprotachykinin 111-129, human; tachyplesin I; xenopsin; and xenopsin 25 (xenin 25), human.

Thyrotropin-releasing hormone (TRH) peptides including, but not limited to, biotinyl-thyrotropin-releasing hormone; [Glu^{1}]-TRH; His-Pro-diketopiperazine; [3-Me-His^{2}]-TRH;

pGlu-Gln-Pro-amide; pGlu-His; [Phe^2]-TRH; prepro TRH 53-74; prepro TRH 83-106; prepro-TRH 160-169 (Ps4, TRH-potentiating peptide); prepro-TRH 178-199; thyrotropin-releasing hormone (TRH); TRH, free acid; TRH-SH Pro; and TRH precursor peptide.

Toxin peptides including, but not limited to, omega-agatoxin TK; agelenin, (spider, Agelena opulenta); apamin (honeybee, Apis mellifera); calcicudine (CaC) (green mamba, Dedroaspis angusticeps); calciseptine (black mamba, Dendroaspis polylepis polylepis); charybdotoxin (ChTX) (scorpion, Leiurus quinquestriatus var. hebraeus); chlorotoxin; conotoxin GI (marine snail, Conus geographus); conotoxin GS (marine snail, Conus geographus); conotoxin MI (Marine Conus magus); alpha-conotoxin EI, Conus ermineus; alpha-conotoxin SIA; alpha-conotoxin ImI; alpha-conotoxin SI (cone snail, Conus striatus); micro-conotoxin GIIIB (marine snail, Conus geographus); omega-conotoxin GVIA (marine snail, Conus geographus); omega-conotoxin MVIIA (Conus magus); omega-conotoxin MVIIC (Conus magus); omega-conotoxin SVIB (cone snail, Conus striatus); endotoxin inhibitor; geographutoxin I (GTX-I) (μ-Conotoxin GIIIA); iberiotoxin (IbTX) (scorpion, Buthus tamulus); kaliotoxin 1-37; kaliotoxin (scorpion, Androct-onus mauretanicus mauretanicus); mast cell-degranulating peptide (MCD-peptide, peptide 401); margatoxin (MgTX) (scorpion, Centruriodes Margaritatus); neurotoxin NSTX-3 (pupua new guinean spider, Nephilia maculata); PLTX-II (spider, Plectreurys tristes); scyllatoxin (leiurotoxin I); and stichodactyla toxin (ShK).

Vasoactive intestinal peptides (VIP/PHI) including, but not limited to, VIP, human, porcine, rat, ovine; VIP-Gly-Lys-Arg-NH2; biotinyl-PHI (biotinyl-PHI-27), porcine; [Glp^{16}] VIP 16-28, porcine; PHI (PHI-27), porcine; PHI (PHI-27), rat; PHM-27 (PHI), human; prepro VIP 81-122, human; preproVIP/PHM 111-122; prepro VIP/PHM 156-170; biotinyl-PHM-27 (biotinyl-PHI), human; vasoactive intestinal contractor (endothelin-beta); vasoactive intestinal octacosa-peptide, chicken; vasoactive intestinal peptide, guinea pig; biotinyl-VIP, human, porcine, rat; vasoactive intestinal peptide 1-12, human, porcine, rat; vasoactive intestinal peptide 10-28, human, porcine, rat; vasoactive intestinal peptide 11-28, human, porcine, rat, ovine; vasoactive intestinal peptide (cod, Gadus morhua); vasoactive intestinal peptide 6-28; vasoactive intestinal peptide antagonist; vasoactive intestinal peptide antagonist ([$Ac-Tyr^1$, $D-Phe^2$]-GHRF 1-29 amide); vasoactive intestinal peptide receptor antagonist (4-Cl-D-Phe^6, Leu^{17}]-VIP); and vasoactive intestinal peptide receptor binding inhibitor, L-8-K.

Vasopressin (ADH) peptides including, but not limited to, vasopressin; [$Asu^{1,6}$,Arg^{8}]-vasopressin; vasotocin; [$Asu^{1,6}$,Arg^{8}]-vasotocin; [Lys^{8}]-vasopressin; pressinoic acid; [Arg^{8}]-desamino vasopressin desglycinamide; [Arg^{8}]-vasopressin (AVP); [Arg^{8}]-vasopressin desglycinamide; biotinyl-[Arg^{8}]-vasopressin (biotinyl-AVP); [D-Arg^{8}]-vasopressin; desamino-[Arg^{8}]-vasopressin; desamino-[D-Arg^{8}]-vasopressin (DDAVP); [deamino-[D-3-(3'-pyridyl-Ala)]-[Arg^{8}]-vasopressin; [1-(beta-Mercapto-beta, beta-cyclopentamethylene propionic acid), 2-(O-methyl)tyrosine]-[Arg^{8}]-vasopressin; vasopressin metabolite neuropeptide [$pGlu^{4}$, Cys^{6}]; vasopressin metabolite neuropeptide [$pGlu^{4}$, Cys^{6}]; [Lys^{8}]-deamino vasopressin desglycinamide; [Lys^{8}]-vasopressin; [Mpr^{1},Val^{4},$DArg^{8}$]-vasopressin; [Phe^{2}, Ile^{3}, Orn^{8}]-vasopressin ([Phe^{2}, Orn^{8}]-vasotocin); [Arg^{8}]-vasotocin; and [d(CH2)5, $Tyr(Me)^{2}$, Orn^{8}]-vasotocin.

Virus related peptides including, but not limited to, fluorogenic human CMV protease substrate; HCV core protein 59-68; HCV NS4A protein 18-40 (JT strain); HCV NS4A protein 21-34 (JT strain); hepatitis B virus receptor binding fragment; hepatitus B virus pre-S region 120-145; [Ala^{127}]-hepatitus B virus pre-S region 120-131; herpes virus inhibitor 2; HIV envelope protein fragment 254-274; HIV gag fragment 129-135; HIV substrate; P 18 peptide; peptide T; [3,5 diiodo-Tyr^{7}] peptide T; R15K HIV-1 inhibitory peptide; T20; T21; V3 decapeptide P 18-110; and virus replication inhibiting peptide.

While certain analogs, fragments, and/or analog fragments of the various polypeptides have been described above, it is to be understood that other analogs, fragments, and/or analog fragments that retain all or some of the activity of the particular polypeptide may also be useful in embodiments of the present invention. Analogs may be obtained by various means, as will be understood by those skilled in the art. For example, certain amino acids may be substituted for other amino acids in a polypeptide without appreciable loss of interactive binding capacity with structures such as, for example, antigen-binding regions of antibodies or binding sites on substrate molecules. As the interactive capacity and nature of a polypeptide drug defines its biological functional activity, certain amino acid sequence substitutions can be made in the amino acid sequence and nevertheless remain a polypeptide with like properties.

In making such substitutions, the hydropathic index of amino acids may be considered. The importance of the hydropathic amino acid index in conferring interactive biologic function on a polypeptide is generally understood in the art. It is accepted that the

relative hydropathic character of the amino acid contributes to the secondary structure of the resultant polypeptide, which in turn defines the interaction of the polypeptide with other molecules, for example, enzymes, substrates, receptors, DNA, antibodies, antigens, and the like. Each amino acid has been assigned a hydropathic index on the basis of its hydrophobicity and charge characteristics as follows: isoleucine (+4.5); valine (+4.2); leucine (+3.8); phenylalanine (+2.8); cysteine/cystine (+2.5); methionine (+1.9); alanine (+1.8); glycine (-0.4); threonine (-0.7); serine (-0.8); tryptophan (-0.9); tyrosine (-1.3); proline (-1.6); histidine (-3.2); glutamate (-3.5); glutamine (-3.5); aspartate (-3.5); asparagine (-3.5); lysine (-3.9); and arginine (-4.5). As will be understood by those skilled in the art, certain amino acids may be substituted by other amino acids having a similar hydropathic index or score and still result in a polypeptide with similar biological activity, *i.e.*, still obtain a biological functionally equivalent polypeptide. In making such changes, the substitution of amino acids whose hydropathic indices are within ±2 of each other is preferred, those which are within ±1 of each other are particularly preferred, and those within ±0.5 of each other are even more particularly preferred.

It is also understood in the art that the substitution of like amino acids can be made effectively on the basis of hydrophilicity. U.S. Patent 4,554,101 provides that the greatest local average hydrophilicity of a protein, as governed by the hydrophilicity of its adjacent amino acids, correlates with a biological property of the protein. As detailed in U.S. Patent 4,554,101, the following hydrophilicity values have been assigned to amino acid residues: arginine (+3.0); lysine (±3.0); aspartate (+3.0 ± 1); glutamate (+3.0 ± 1); seine (+0.3); asparagine (+0.2); glutamine (+0.2); glycine (0); threonine (-0.4); proline (-0.5 ± 1); alanine (-0.5); histidine (-0.5); cysteine (-1.0); methionine (-1.3); valine (-1.5); leucine (-1.8); isoleucine (-1.8); tyrosine (-2.3); phenylalanine (-2.5); tryptophan (-3.4). As is understood by those skilled in the art, an amino acid can be substituted for another having a similar hydrophilicity value and still obtain a biologically equivalent, and in particular, an immunologically equivalent polypeptide. In such changes, the substitution of amino acids whose hydrophilicity values are within ±2 of each other is preferred, those which are within ±1 of each other are particularly preferred, and those within ±0.5 of each other are even more particularly preferred.

As outlined above, amino acid substitutions are generally therefore based on the relative similarity of the amino acid side-chain substituents, for example, their

hydrophobicity, hydrophilicity, charge, size, and the like. Exemplary substitutions (i.e., amino acids that may be interchanged without significantly altering the biological activity of the polypeptide) that take various of the foregoing characteristics into consideration are well known to those of skill in the art and include, for example: arginine and lysine; glutamate and aspartate; serine and threonine; glutamine and asparagine; and valine, leucine and isoleucine.

In embodiments of the present invention, a substantially monodispersed mixture of drug-oligomer conjugates is provided. Preferably, at least about 96, 97, 98 or 99 percent of the conjugates in the mixture have the same molecular weight. More preferably,, the mixture is a monodispersed mixture. Even more preferably, the mixture is a substantially purely monodispersed mixture of drug-oligomer conjugates. Still more preferably, at least about 96, 97, 98 or 99 percent of the conjugates in the mixture have the same molecular weight and the same molecular structure. Most preferably, the mixture is a purely monodispersed mixture.

The oligomer may be various oligomers comprising a polyalkylene glycol moiety as will be understood by those skilled in the art. Preferably, the polyalkylene glycol moiety has at least 2, 3, or 4 polyalkylene glycol subunits. More preferably, the polyalkylene glycol moiety has at least 5 or 6 polyalkylene glycol subunits. Most preferably, the polyalkylene glycol moiety of the oligomer has at least 7 polyalkylene glycol subunits. The polyalkylene glycol moiety of the oligomer is preferably a lower alkyl polyalkylene glycol moiety such as a polyethylene glycol moiety, a polypropylene glycol moiety, or a polybutylene glycol moiety. When the polyalkylene moiety is a polypropylene glycol moiety, the polypropylene glycol moiety preferably has a uniform structure. An exemplary polypropylene glycol moiety having a uniform structure is as follows:

$$-O-\underset{\displaystyle CH_3}{\underset{|}{CH}}-CH_2-O-\underset{\displaystyle CH_3}{\underset{|}{CH}}-CH_2-O-\underset{\displaystyle CH_3}{\underset{|}{CH}}-CH_2-O-\underset{\displaystyle CH_3}{\underset{|}{CH}}-CH_2-O-$$

This uniform polypropylene glycol structure may be described as having only one methyl substituted carbon atom adjacent each oxygen atom in the polypropylene glycol chain. Such uniform polypropylene glycol moieties may exhibit both lipophilic and hydrophilic characteristics and thus be useful in providing amphiphilic growth hormone drug-oligomer conjugates without the use of lipophilic polymer moieties. Furthermore, coupling the secondary alcohol moiety of the polypropylene glycol moiety with a drug may provide the drug (e.g., a polypeptide) with improved resistance to degradation caused by enzymes such as trypsin and chymotrypsin found, for example, in the gut.

Uniform polypropylene glycol according to embodiments of the present invention is preferably synthesized as illustrated in **Figures 11** through **13**, which will now be described. As illustrated in **Figure 11**, 1,2-propanediol **53** is reacted with a primary alcohol blocking reagent to provide a secondary alcohol extension monomer **54**. The primary alcohol blocking reagent may be various primary alcohol blocking reagents as will be understood by those skilled in the art including, but not limited to, silylchloride compounds such as t-butyldiphenylsilylchloride and t-butyldimethylsilylchloride, and esterification reagents such as Ac_2O. Preferably, the primary alcohol blocking reagent is a primary alcohol blocking reagent that is substantially non-reactive with secondary alcohols, such as t-butyldiphenylsilylchloride or t-butyldimethylsilylchloride. The secondary alcohol extension monomer (**54**) may be reacted with methanesulfonyl chloride ($MeSO_2Cl$) to provide a primary extension alcohol monomer mesylate **55**.

Alternatively, the secondary alcohol extension monomer **54** may be reacted with a secondary alcohol blocking reagent to provide compound **56**. The secondary alcohol blocking reagent may be various secondary alcohol blocking reagents as will be understood by those skilled in the art including, but not limited to, benzyl chloride. The compound **56** may be reacted with a B_1 de-blocking reagent to remove the blocking moiety B_1 and provide a primary alcohol extension monomer **57**. The B_1 de-blocking reagent may be selected from various de-blocking reagents as will be understood by one skilled in the art. When the primary alcohol has been blocked by forming an ester, the B_1 de-blocking reagent is a de-esterification reagent, such as a base (e.g., potassium carbonate). When the primary alcohol has been blocked using a silylchloride, the B_1 de-blocking reagent is preferably tetrabutylammonium fluoride (TBAF). The primary alcohol extension monomer **57** may be reacted with methane sulfonyl chloride to provide a secondary alcohol extension monomer mesylate **58**.

The primary alcohol extension monomer **54** and the secondary alcohol extension monomer **57** may be capped as follows. The secondary alcohol extension monomer **54** may be reacted with a capping reagent to provide a compound **59**. The capping reagent may be various capping reagents as will be understood by those skilled in the art including, but not limited to, alkyl halides such as methyl chloride. The compound **59** may be reacted with a B_1 de-blocking agent as described above to provide a primary alcohol capping monomer **60**. The primary alcohol capping monomer **60** may be reacted with methane sulfonyl chloride to

provide the secondary alcohol capping monomer mesylate **61**. The primary alcohol extension monomer **57** may be reacted with a capping reagent to provide a compound **62**. The capping reagent may be various capping reagents as described above. The compound **62** may be reacted with a B_2 de-blocking reagent to remove the blocking moiety B_2 and provide a secondary alcohol capping monomer **63**. The B_2 de-blocking reagent may be various de-blocking agents as will be understood by those skilled in the art including, but not limited to, H_2 in the presence of a palladium/activated carbon catalyst. The secondary alcohol capping monomer may be reacted with methanesulfonyl chloride to provide a primary alcohol capping monomer mesylate **64**. While the embodiments illustrated in **Figure 11** show the synthesis of capping monomers, it is to be understood that similar reactions may be performed to provide capping polymers.

In general, chain extensions may be effected by reacting a primary alcohol extension mono- or poly-mer such as the primary alcohol extension monomer **57** with a primary alcohol extension mono- or poly-mer mesylate such as the primary alcohol extension monomer mesylate **55** to provide various uniform polypropylene chains or by reacting a secondary alcohol extension mono- or poly-mer such as the secondary alcohol extension monomer **54** with a secondary alcohol extension mono-or poly-mer mesylate such as the secondary alcohol extension monomer mesylate **58**.

For example, in **Figure 13**, the primary alcohol extension monomer mesylate **55** is reacted with the primary alcohol extension monomer **57** to provide a dimer compound **65**. Alternatively, the secondary alcohol extension monomer mesylate **58** may be reacted with the secondary alcohol extension monomer **54** to provide the dimer compound **65**. The B_1 blocking moiety on the dimer compound **65** may be removed using a B_1 de-blocking reagent as described above to provide a primary alcohol extension dimer **66**. The primary alcohol extension dimer **66** may be reacted with methane sulfonyl chloride to provide a secondary alcohol extension dimer mesylate **67**. Alternatively, the B_2 blocking moiety on the dimer compound **65** may be removed using the B_2 de-blocking reagent as described above to provide a secondary alcohol extension dimer **69**. The secondary alcohol extension dimer **69** may be reacted with methane sulfonyl chloride to provide a primary alcohol extension dimer mesylate **70**.

As will be understood by those skilled in the art, the chain extension process may be repeated to achieve various other chain lengths. For example, as illustrated in **Figure 13**, the

primary alcohol extension dimer **66** may be reacted with the primary alcohol extension dimer mesylate **70** to provide a tetramer compound **72**. As further illustrated in **Figure 13**, a generic chain extension reaction scheme involves reacting the primary alcohol extension mono- or poly-mer **73** with the primary alcohol extension mono- or poly-mer mesylate **74** to provide the uniform polypropylene polymer **75**. The values of m and n may each range from 0 to 1000 or more. Preferably, m and n are each from 0 to 50. While the embodiments illustrated in **Figure 13** show primary alcohol extension mono- and/or poly-mers being reacted with primary alcohol extension mono- and/or poly-mer mesylates, it is to be understood that similar reactions may be carried out using secondary alcohol extension mono- and/or poly-mers and secondary alcohol extension mono- and/or poly-mer mesylates.

An end of a primary alcohol extension mono- or poly-mer or an end of a primary alcohol extension mono- or poly-mer mesylate may be reacted with a primary alcohol capping mono- or poly-mer mesylate or a primary alcohol capping mono- or poly-mer, respectively, to provide a capped uniform polypropylene chain. For example, as illustrated in **Figure 12**, the primary alcohol extension dimer mesylate **70** is reacted with the primary alcohol capping monomer **60** to provide the capped/blocked primary alcohol extension trimer **71**. As will be understood by those skilled in the art, the B_1 blocking moiety may be removed and the resulting capped primary alcohol extension trimer may be reacted with a primary alcohol extension mono- or poly-mer mesylate to extend the chain of the capped trimer **71**.

An end of a secondary alcohol extension mono-or poly-mer or an end of a secondary alcohol extension mono-or poly-mer mesylate may be reacted with a secondary alcohol capping mono-or poly-mer mesylate or a secondary alcohol capping mono- or poly-mer, respectively, to provide a capped uniform polypropylene chain. For example, as illustrated in **Figure 12**, the secondary alcohol extension dimer mesylate **67** is reacted with the secondary alcohol capping monomer **63** to provide the capped/blocked primary alcohol extension trimer **68**. The B_2 blocking moiety may be removed as described above and the resulting capped secondary alcohol extension trimer may be reacted with a secondary alcohol extension mer mesylate to extend the chain of the capped trimer **68**. While the syntheses illustrated in **Figure 12** show the reaction of a dimer with a capping monomer to provide a trimer, it is to be understood that the capping process may be performed at any point in the synthesis of a uniform polypropylene glycol moiety, or, alternatively, uniform polypropylene glycol moieties may be provided that are not capped. While the embodiments illustrated in **Figure**

12 show the capping of a polybutylene oligomer by synthesis with a capping monomer, it is to be understood that polybutylene oligomers of the present invention may be capped directly (i.e., without the addition of a capping monomer) using a capping reagent as described above in **Figure 11**.

Uniform polypropylene glycol moieties according to embodiments of the present invention may be coupled to a drug, a lipophilic moiety such as a carboxylic acid, and/or various other moieties by various methods as will be understood by those skilled in the art including, but not limited to, those described herein with respect to polyethylene glycol moieties.

The oligomer may comprise one or more other moieties as will be understood by those skilled in the art including, but not limited to, hydrophilic moieties, lipophilic moieties, spacer moieties, linker moieties, and terminating moieties. The various moieties in the oligomer are covalently coupled to one another by either hydrolyzable or non-hydrolyzable bonds.

The oligomer may further comprise one or more hydrophilic moieties including, but not limited to, sugars, polyalkylene glycols, and polyamine/PEG copolymers. Adjacent polyalkylene glycol moieties will be considered to be the same moiety if they are coupled by an ether bond and have the same alkyl structure. For example, the moiety

$$—O—C_2H_4—O—C_2H_4—O—C_2H_4—O—C_2H_4—O—C_2H_4—O—C_2H_4—$$

is a single polyethylene glycol moiety having six polyethylene glycol subunits. Adjacent polyalkylene glycol moieties will be considered to be different moieties if they are coupled by a bond other than an ether bond or if they have different alkyl structures. For example, the moiety

$$—O—C_2H_4—O—C_2H_4—O—C_2H_4—O—C_2H_4—\overset{\overset{\displaystyle O}{\|}}{C}—O—C_2H_4—O—C_2H_4—$$

is a polyethylene glycol moiety having four polyethylene glycol subunits and a hydrophilic moiety having two polyethylene glycol subunits. Preferably, oligomers according to embodiments of the present invention comprise a polyalkylene glycol moiety and do not further comprise a hydrophilic moiety.

The oligomer may further comprise one or more lipophilic moieties as will be understood by those skilled in the art. The lipophilic moiety is preferably a saturated or unsaturated, linear or branched alkyl moiety or a saturated or unsaturated, linear or branched

fatty acid moiety. When the lipophilic moiety is an alkyl moiety, it is preferably a linear, saturated or unsaturated alkyl moiety having 1 to 28 carbon atoms. More preferably, the alkyl moiety has 2 to 12 carbon atoms. When the lipophilic moiety is a fatty acid moiety, it is preferably a natural fatty acid moiety that is linear, saturated or unsaturated, having 2 to 18 carbon atoms. More preferably, the fatty acid moiety has 3 to 14 carbon atoms. Most preferably, the fatty acid moiety has at least 4, 5 or 6 carbon atoms.

The oligomer may further comprise one or more spacer moieties as will be understood by those skilled in the art. Spacer moieties may, for example, be used to separate a hydrophilic moiety from a lipophilic moiety, to separate a lipophilic moiety or hydrophilic moiety from the drug, to separate a first hydrophilic or lipophilic moiety from a second hydrophilic or lipophilic moiety, or to separate a hydrophilic moiety or lipophilic moiety from a linker moiety. Spacer moieties are preferably selected from the group consisting of sugar, cholesterol and glycerine moieties.

The oligomer may further comprise one or more linker moieties that are used to couple the oligomer with the drug as will be understood by those skilled in the art. Linker moieties are preferably selected from the group consisting of alkyl and fatty acid moieties.

The oligomer may further comprise one or more terminating moieties at the one or more ends of the oligomer which are not coupled to the drug. The terminating moiety is preferably an alkyl or alkoxy moiety, and is more preferably a lower alkyl or lower alkoxy moiety. Most preferably, the terminating moiety is methyl or methoxy. While the terminating moiety is preferably an alkyl or alkoxy moiety, it is to be understood that the terminating moiety may be various moieties as will be understood by those skilled in the art including, but not limited to, sugars, cholesterol, alcohols, and fatty acids.

The oligomer is preferably covalently coupled to the drug. In some embodiments, the drug is coupled to the oligomer utilizing a hydrolyzable bond (e.g., an ester or carbonate bond). A hydrolyzable coupling may provide a drug-oligomer conjugate that acts as a prodrug. In certain instances, for example where the drug-oligomer conjugate is inactive (i.e., the conjugate lacks the ability to affect the body through the drug's primary mechanism of action), a hydrolyzable coupling may provide for a time-release or controlled-release effect, administering the drug over a given time period as one or more oligomers are cleaved from their respective drug-oligomer conjugates to provide the active drug. In other embodiments, the drug is coupled to the oligomer utilizing a non-hydrolyzable bond (e.g., a

carbamate, amide, or ether bond). Use of a non-hydrolyzable bond may be preferable when it is desirable to allow the drug-oligomer conjugate to circulate in the bloodstream for an extended period of time, preferably at least 2 hours.

While the oligomer is preferably covalently coupled to the drug, it is to be understood that the oligomer may be non-covalently coupled to the drug to form a non-covalently conjugated drug-oligomer complex. As will be understood by those skilled in the art, non-covalent couplings include, but are not limited to, hydrogen bonding, ionic bonding, Van der Waals bonding, and micellular or liposomal encapsulation. According to embodiments of the present invention, oligomers may be suitably constructed, modified and/or appropriately functionalized to impart the ability for non-covalent conjugation in a selected manner (e.g., to impart hydrogen bonding capability), as will be understood by those skilled in the art. According to other embodiments of present invention, oligomers may be derivatized with various compounds including, but not limited to, amino acids, oligopeptides, peptides, bile acids, bile acid derivatives, fatty acids, fatty acid derivatives, salicylic acids, salicylic acid derivatives, aminosalicylic acids, and aminosalicylic acid derivatives. The resulting oligomers can non-covalently couple (complex) with drug molecules, pharmaceutical products, and/or pharmaceutical excipients. The resulting complexes preferably have balanced lipophilic and hydrophilic properties. According to still other embodiments of the present invention, oligomers may be derivatized with amine and/or alkyl amines. Under suitable acidic conditions, the resulting oligomers can form non-covalently conjugated complexes with drug molecules, pharmaceutical products and/or pharmaceutical excipients. The products resulting from such complexation preferably have balanced lipophilic and hydrophilic properties.

More than one oligomer (i.e., a plurality of oligomers) may be coupled to the drug. The oligomers in the plurality are preferably the same. However, it is to be understood that the oligomers in the plurality may be different from one another, or, alternatively, some of the oligomers in the plurality may be the same and some may be different. When a plurality of oligomers are coupled to the drug, it may be preferable to couple one or more of the oligomers to the drug with hydrolyzable bonds and couple one or more of the oligomers to the drug with non-hydrolyzable bonds. Alternatively, all of the bonds coupling the plurality of oligomers to the drug may be hydrolyzable, but have varying degrees of hydrolyzability such that, for example, one or more of the oligomers is rapidly removed from the drug by

hydrolysis in the body and one or more of the oligomers is slowly removed from the drug by hydrolysis in the body.

The oligomer may be coupled to the drug at various nucleophilic residues of the drug including, but not limited to, nucleophilic hydroxyl functions and/or amino functions. When the drug is a polypeptide, a nucleophilic hydroxyl function may be found, for example, at serine and/or tyrosine residues, and a nucleophilic amino function may be found, for example, at histidine and/or lysine residues, and/or at the one or more N-termini of the polypeptide. When an oligomer is coupled to the one or more N-termini of the polypeptide, the coupling preferably forms a secondary amine. For example, when the drug is human insulin, the oligomer may be coupled to an amino functionality of the insulin including the amino functionality of Gly^{A1}, the amino functionality of Phe^{B1}, and the amino functionality of Lys^{B29}. When one oligomer is coupled to the human insulin, the oligomer is preferably coupled to the amino functionality of Lys^{B29}. When two oligomers are coupled to the human insulin, the oligomers are preferably coupled to the amino functionality of Phe^{B1} and the amino functionality of Lys^{B29}. While more than one oligomer may be coupled to the human insulin, a higher activity (improved glucose lowering ability) is observed for the mono-conjugated human insulin. As another example, when the drug is salmon calcitonin, the oligomer may be coupled to an amino functionality of the salmon calcitonin, including the amino functionality of Lys^{11}, Lys^{18} and the N-terminus. While one or more oligomers may be coupled to the salmon calcitonin, a higher activity (improved glucose lowering ability) is observed for the di-conjugated salmon calcitonin where an oligomer is coupled to the amino functionality of Lys^{11} and an oligomer is coupled to the amino functionality of Lys^{18}. As yet another example, when the drug is human growth hormone, the oligomer may be coupled to an amino functionality of Phe^{1}, Lys^{38}, Lys^{41}, Lys^{70}, Lys^{115}, Lys^{140}, Lys^{145}, Lys^{158}, Lys^{168}, and/or Lys^{172}.

Substantially monodispersed mixtures of drug-oligomer conjugates of the present invention may be synthesized by various methods. For example, a substantially monodispersed mixture of oligomers consisting of carboxylic acid and polyethylene glycol is synthesized by contacting a substantially monodispersed mixture of carboxylic acid with a substantially monodispersed mixture of polyethylene glycol under conditions sufficient to provide a substantially monodispersed mixture of oligomers. The oligomers of the substantially monodispersed mixture are then activated so that they are capable of reacting

with a drug to provide a drug-oligomer conjugate. One embodiment of a synthesis route for providing a substantially monodispersed mixture of activated oligomers is illustrated in **Figure 3** and described in Examples 11-18 hereinbelow. Another embodiment of a synthesis route for providing a substantially monodispersed mixture of activated oligomers is illustrated in **Figure 4** and described in Examples 19-24 hereinbelow. Still another embodiment of a synthesis route for providing a substantially monodispersed mixture of activated oligomers is illustrated in **Figure 5** and described in Examples 25-29 hereinbelow. Yet another embodiment of a synthesis route for providing a substantially monodispersed mixture of activated oligomers is illustrated in **Figure 6** and described in Examples 30-31 hereinbelow. Another embodiment of a synthesis route for providing a substantially monodispersed mixture of activated oligomers is illustrated in **Figure 7** and described in Examples 32-37 hereinbelow. Still another embodiment of a synthesis route for providing a substantially monodispersed mixture of activated oligomers is illustrated in **Figure 8** and described in Example 38 hereinbelow. Yet another embodiment of a synthesis route for providing a substantially monodispersed mixture of activated oligomers is illustrated in **Figure 9** and described in Example 39 hereinbelow. Another embodiment of a synthesis route for providing a substantially monodispersed mixture of activated oligomers is illustrated in **Figure 10** and described in Example 40 hereinbelow.

The substantially monodispersed mixture of activated oligomers may be reacted with a substantially monodispersed mixture of drugs under conditions sufficient to provide a mixture of drug-oligomer conjugates, as described, for example, in Examples 41-120 hereinbelow. As will be understood by those skilled in the art, the reaction conditions (e.g., selected molar ratios, solvent mixtures and/or pH) may be controlled such that the mixture of drug-oligomer conjugates resulting from the reaction of the substantially monodispersed mixture of activated oligomers and the substantially monodispersed mixture of drugs is a substantially monodispersed mixture. For example, conjugation at the amino functionality of lysine may be suppressed by maintaining the pH of the reaction solution below the pK_a of lysine. Alternatively, the mixture of drug-oligomer conjugates may be separated and isolated utilizing, for example, HPLC to provide a substantially monodispersed mixture of drug-oligomer conjugates, for example mono-, di-, or tri-conjugates. The degree of conjugation (e.g., whether the isolated molecule is a mono-, di-, or tri-conjugate) of a particular isolated conjugate may be determined and/or verified utilizing various techniques as will be

understood by those skilled in the art including, but not limited to, mass spectroscopy. The particular conjugate structure (e.g., whether the oligomer is at Gly^{A1}, Phe^{B1}, or Lys^{B29} of a human insulin monoconjugate) may be determined and/or verified utilizing various techniques as will be understood by those skilled in the art including, but not limited to, sequence analysis, peptide mapping, selective enzymatic cleavage, and/or endopeptidase cleavage.

As will be understood by those skilled in the art, one or more of the reaction sites on the drug may be blocked by, for example, reacting the drug with a suitable blocking reagent such as N-tert-butoxycarbonyl (t-BOC), or N-(9-fluorenylmethoxycarbonyl) (N-FMOC). This process may be preferred, for example, when the drug is a polypeptide and it is desired to form an unsaturated conjugate (i.e., a conjugate wherein not all nucleophilic residues are conjugated) having an oligomer at one or more of the N-termini of the polypeptide. Following such blocking, the substantially monodispersed mixture of blocked drugs may be reacted with the substantially monodispersed mixture of activated oligomers to provide a mixture of drug-oligomer conjugates having oligomer(s) coupled to one or more nucleophilic residues and having blocking moieties coupled to other nucleophilic residues. After the conjugation reaction, the drug-oligomer conjugates may be de-blocked as will be understood by those skilled in the art. If necessary, the mixture of drug-oligomer conjugates may then be separated as described above to provide a substantially monodispersed mixture of drug-oligomer conjugates. Alternatively, the mixture of drug-oligomer conjugates may be separated prior to de-blocking.

Substantially monodispersed mixtures of drug-oligomer conjugates according to embodiments of the present invention preferably have improved properties when compared with those of conventional mixtures. For example, a substantially monodispersed mixture of drug-oligomer conjugates preferably has an *in vivo* activity that is greater than the *in vivo* activity of a polydispersed mixture of drug-oligomer conjugates having the same number average molecular weight as the substantially monodispersed mixture. As will be understood by those skilled in the art, the number average molecular weight of the substantially monodispersed mixture and the number average weight of the polydispersed mixture may be measured by various methods including, but not limited to, size exclusion chromatography such as gel permeation chromatography as described, for example, in H.R. Allcock & F.W. Lampe, CONTEMPORARY POLYMER CHEMISTRY 394-402 (2d. ed., 1991).

As another example, a substantially monodispersed mixture of drug-oligomer conjugates preferably has an *in vitro* activity that is greater than the *in vitro* activity of a polydispersed mixture of drug-oligomer conjugates having the same number average molecular weight as the substantially monodispersed mixture. As will be understood by those skilled in the art, the number average molecular weight of the substantially monodispersed mixture and the number average weight of the polydispersed mixture may be measured by various methods including, but not limited to, size exclusion chromatography such as gel permeation chromatography. The *in vitro* activity of a particular mixture may be measured by various methods, as will be understood by those skilled in the art. Preferably, the *in vitro* activity is measured using a Cytosensor® Microphysiometer commercially available from Molecular Devices Corporation of Sunnyvale, California. The microphysiometer monitors small changes in the rates of extracellular acidification in response to a drug being added to cultured cells in a transwell. This response is proportional to the activity of the molecule under study.

As still another example, a substantially monodispersed mixture of drug-oligomer conjugates preferably has an increased resistance to degradation by chymotrypsin when compared to the resistance to degradation by chymotrypsin of a polydispersed mixture of drug-oligomer conjugates having the same number average molecular weight as the substantially monodispersed mixture. As will be understood by those skilled in the art, the number average molecular weight of the substantially monodispersed mixture and the number average weight of the polydispersed mixture may be measured by various methods including, but not limited to, size exclusion chromatography.

As yet another example, a substantially monodispersed mixture of drug-oligomer conjugates preferably has an inter-subject variability that is less than the inter-subject variability of a polydispersed mixture of drug-oligomer conjugates having the same number average molecular weight as the substantially monodispersed mixture. As will be understood by those skilled in the art, the number average molecular weight of the substantially monodispersed mixture and the number average weight of the polydispersed mixture may be measured by various methods including, but not limited to, size exclusion chromatography. The inter-subject variability may be measured by various methods as will be understood by those skilled in the art. The inter-subject variability is preferably calculated as follows. The area under a dose response curve (AUC) (i.e., the area between the dose-response curve and a

baseline value) is determined for each subject. The average AUC for all subjects is determined by summing the AUCs of each subject and dividing the sum by the number of subjects. The absolute value of the difference between the subject's AUC and the average AUC is then determined for each subject. The absolute values of the differences obtained are then summed to give a value that represents the inter-subject variability. Lower values represent lower inter-subject variabilities and higher values represent higher inter-subject variabilities.

Substantially monodispersed mixtures of drug-oligomer conjugates according to embodiments of the present invention preferably have two or more of the above-described improved properties. More preferably, substantially monodispersed mixtures of drug-oligomer conjugates according to embodiments of the present invention have three or more of the above-described improved properties. Most preferably, substantially monodispersed mixtures of drug-oligomer conjugates according to embodiments of the present invention have all four of the above-described improved properties.

In still other embodiments according to the present invention, a mixture of conjugates having a molecular weight distribution with a standard deviation of less than about 22 Daltons is provided. Each conjugate in the mixture includes a drug coupled to an oligomer that comprises a polyalkylene glycol moiety. The standard deviation is preferably less than about 14 Daltons and is more preferably less than about 11 Daltons. The molecular weight distribution may be determined by methods known to those skilled in the art including, but not limited to, size exclusion chromatography such as gel permeation chromatography as described, for example, in H.R. Allcock & F.W. Lampe, CONTEMPORARY POLYMER CHEMISTRY 394-402 (2d. ed., 1991). The standard deviation of the molecular weight distribution may then be determined by statistical methods as will be understood by those skilled in the art.

The oligomer may be various oligomers comprising a polyalkylene glycol moiety as will be understood by those skilled in the art. Preferably, the polyalkylene glycol moiety has at least 2, 3, or 4 polyalkylene glycol subunits. More preferably, the polyalkylene glycol moiety has at least 5 or 6 polyalkylene glycol subunits. Most preferably, the polyalkylene glycol moiety of the oligomer has at least 7 polyalkylene glycol subunits. The polyalkylene glycol moiety of the oligomer is preferably a lower alkyl polyalkylene glycol moiety such as a polyethylene glycol moiety, a polypropylene glycol moiety, or a polybutylene glycol

moiety. When the polyalkylene moiety is a polypropylene glycol moiety, the polypropylene glycol moiety preferably has a uniform structure. An exemplary polypropylene glycol moiety having a uniform structure is as follows:

$$-O-\underset{\displaystyle CH_3}{\underset{|}{CH}}-CH_2-O-\underset{\displaystyle CH_3}{\underset{|}{CH}}-CH_2-O-\underset{\displaystyle CH_3}{\underset{|}{CH}}-CH_2-O-\underset{\displaystyle CH_3}{\underset{|}{CH}}-CH_2-O-$$

This uniform polypropylene glycol structure may be described as having only one methyl substituted carbon atom adjacent each oxygen atom in the polypropylene glycol chain. Such uniform polypropylene glycol moieties may exhibit both lipophilic and hydrophilic characteristics and thus be useful in providing amphiphilic drug-oligomer conjugates without the use of lipophilic polymer moieties. Furthermore, coupling the secondary alcohol moiety of the polypropylene glycol moiety with a drug may provide the drug (e.g., a polypeptide) with improved resistance to degradation caused by enzymes such as trypsin and chymotrypsin found, for example, in the gut.

Uniform polypropylene glycol according to embodiments of the present invention is preferably synthesized as illustrated in **Figures 11** through **13**, which will now be described. As illustrated in **Figure 11**, 1,2-propanediol **53** is reacted with a primary alcohol blocking reagent to provide a secondary alcohol extension monomer **54**. The primary alcohol blocking reagent may be various primary alcohol blocking reagents as will be understood by those skilled in the art including, but not limited to, silylchloride compounds such as t-butyldiphenylsilylchloride and t-butyldimethylsilylchloride, and esterification reagents such as Ac_2O. Preferably, the primary alcohol blocking reagent is a primary alcohol blocking reagent that is substantially non-reactive with secondary alcohols, such as t-butyldiphenylsilylchloride or t-butyldimethylsilylchloride. The secondary alcohol extension monomer (**54**) may be reacted with methanesulfonyl chloride ($MeSO_2Cl$) to provide a primary extension alcohol monomer mesylate **55**.

Alternatively, the secondary alcohol extension monomer **54** may be reacted with a secondary alcohol blocking reagent to provide compound **56**. The secondary alcohol blocking reagent may be various secondary alcohol blocking reagents as will be understood by those skilled in the art including, but not limited to, benzyl chloride. The compound **56** may be reacted with a B_1 de-blocking reagent to remove the blocking moiety B_1 and provide a primary alcohol extension monomer **57**. The B_1 de-blocking reagent may be selected from various de-blocking reagents as will be understood by one skilled in the art. When the

primary alcohol has been blocked by forming an ester, the B_1 de-blocking reagent is a de-esterification reagent, such as a base (e.g., potassium carbonate). When the primary alcohol has been blocked using a silylchloride, the B_1 de-blocking reagent is preferably tetrabutylammonium fluoride (TBAF). The primary alcohol extension monomer **57** may be reacted with methane sulfonyl chloride to provide a secondary alcohol extension monomer mesylate **58**.

The primary alcohol extension monomer **54** and the secondary alcohol extension monomer **57** may be capped as follows. The secondary alcohol extension monomer **54** may be reacted with a capping reagent to provide a compound **59**. The capping reagent may be various capping reagents as will be understood by those skilled in the art including, but not limited to, alkyl halides such as methyl chloride. The compound **59** may be reacted with a B_1 de-blocking agent as described above to provide a primary alcohol capping monomer **60**. The primary alcohol capping monomer **60** may be reacted with methane sulfonyl chloride to provide the secondary alcohol capping monomer mesylate **61**. The primary alcohol extension monomer **57** may be reacted with a capping reagent to provide a compound **62**. The capping reagent may be various capping reagents as described above. The compound **62** may be reacted with a B_2 de-blocking reagent to remove the blocking moiety B_2 and provide a secondary alcohol capping monomer **63**. The B_2 de-blocking reagent may be various de-blocking agents as will be understood by those skilled in the art including, but not limited to, H_2 in the presence of a palladium/activated carbon catalyst. The secondary alcohol capping monomer may be reacted with methanesulfonyl chloride to provide a primary alcohol capping monomer mesylate **64**. While the embodiments illustrated in **Figure 11** show the synthesis of capping monomers, it is to be understood that similar reactions may be performed to provide capping polymers.

In general, chain extensions may be effected by reacting a primary alcohol extension mono- or poly-mer such as the primary alcohol extension monomer **57** with a primary alcohol extension mono- or poly-mer mesylate such as the primary alcohol extension monomer mesylate **55** to provide various uniform polypropylene chains or by reacting a secondary alcohol extension mono- or poly-mer such as the secondary alcohol extension monomer **54** with a secondary alcohol extension mono-or poly-mer mesylate such as the secondary alcohol extension monomer mesylate **58**.

For example, in **Figure 13**, the primary alcohol extension monomer mesylate **55** is reacted with the primary alcohol extension monomer **57** to provide a dimer compound **65**. Alternatively, the secondary alcohol extension monomer mesylate **58** may be reacted with the secondary alcohol extension monomer **54** to provide the dimer compound **65**. The B_1 blocking moiety on the dimer compound **65** may be removed using a B_1 de-blocking reagent as described above to provide a primary alcohol extension dimer **66**. The primary alcohol extension dimer **66** may be reacted with methane sulfonyl chloride to provide a secondary alcohol extension dimer mesylate **67**. Alternatively, the B_2 blocking moiety on the dimer compound **65** may be removed using the B_2 de-blocking reagent as described above to provide a secondary alcohol extension dimer **69**. The secondary alcohol extension dimer **69** may be reacted with methane sulfonyl chloride to provide a primary alcohol extension dimer mesylate **70**.

As will be understood by those skilled in the art, the chain extension process may be repeated to achieve various other chain lengths. For example, as illustrated in **Figure 13**, the primary alcohol extension dimer **66** may be reacted with the primary alcohol extension dimer mesylate **70** to provide a tetramer compound **72**. As further illustrated in **Figure 13**, a generic chain extension reaction scheme involves reacting the primary alcohol extension mono- or poly-mer **73** with the primary alcohol extension mono- or poly-mer mesylate **74** to provide the uniform polypropylene polymer **75**. The values of m and n may each range from 0 to 1000 or more. Preferably, m and n are each from 0 to 50. While the embodiments illustrated in **Figure 13** show primary alcohol extension mono- and/or poly-mers being reacted with primary alcohol extension mono- and/or poly-mer mesylates, it is to be understood that similar reactions may be carried out using secondary alcohol extension mono- and/or poly-mers and secondary alcohol extension mono- and/or poly-mer mesylates.

An end of a primary alcohol extension mono- or poly-mer or an end of a primary alcohol extension mono- or poly-mer mesylate may be reacted with a primary alcohol capping mono- or poly-mer mesylate or a primary alcohol capping mono- or poly-mer, respectively, to provide a capped uniform polypropylene chain. For example, as illustrated in **Figure 12**, the primary alcohol extension dimer mesylate **70** is reacted with the primary alcohol capping monomer **60** to provide the capped/blocked primary alcohol extension trimer **71**. As will be understood by those skilled in the art, the B_1 blocking moiety may be removed

and the resulting capped primary alcohol extension trimer may be reacted with a primary alcohol extension mono- or poly-mer mesylate to extend the chain of the capped trimer **71**.

An end of a secondary alcohol extension mono-or poly-mer or an end of a secondary alcohol extension mono-or poly-mer mesylate may be reacted with a secondary alcohol capping mono-or poly-mer mesylate or a secondary alcohol capping mono- or poly-mer, respectively, to provide a capped uniform polypropylene chain. For example, as illustrated in **Figure 12**, the secondary alcohol extension dimer mesylate **67** is reacted with the secondary alcohol capping monomer **63** to provide the capped/blocked primary alcohol extension trimer **68**. The B_2 blocking moiety may be removed as described above and the resulting capped secondary alcohol extension trimer may be reacted with a secondary alcohol extension mer mesylate to extend the chain of the capped trimer **68**. While the syntheses illustrated in **Figure 12** show the reaction of a dimer with a capping monomer to provide a trimer, it is to be understood that the capping process may be performed at any point in the synthesis of a uniform polypropylene glycol moiety, or, alternatively, uniform polypropylene glycol moieties may be provided that are not capped. While the embodiments illustrated in **Figure 12** show the capping of a polybutylene oligomer by synthesis with a capping monomer, it is to be understood that polybutylene oligomers of the present invention may be capped directly (i.e., without the addition of a capping monomer) using a capping reagent as described above in **Figure 11**.

Uniform polypropylene glycol moieties according to embodiments of the present invention may be coupled to a drug, a lipophilic moiety such as a carboxylic acid, and/or various other moieties by various methods as will be understood by those skilled in the art including, but not limited to, those described herein with respect to polyethylene glycol moieties.

The oligomer may comprise one or more other moieties as will be understood by those skilled in the art including, but not limited to, hydrophilic moieties, lipophilic moieties, spacer moieties, linker moieties, and terminating moieties. The various moieties in the oligomer are covalently coupled to one another by either hydrolyzable or non-hydrolyzable bonds.

The oligomer may further comprise one or more hydrophilic moieties including, but not limited to, sugars, polyalkylene glycols, and polyamine/PEG copolymers. Adjacent

polyalkylene glycol moieties will be considered to be the same moiety if they are coupled by an ether bond and have the same alkyl structure. For example, the moiety

$$—O—C_2H_4—O—C_2H_4—O—C_2H_4—O—C_2H_4—O—C_2H_4—O—C_2H_4—$$

is a single polyethylene glycol moiety having six polyethylene glycol subunits. Adjacent polyalkylene glycol moieties will be considered to be different moieties if they are coupled by a bond other than an ether bond or if they have different alkyl structures. For example, the moiety

$$—O—C_2H_4—O—C_2H_4—O—C_2H_4—O—C_2H_4—\overset{\displaystyle O}{\overset{\|}{C}}—O—C_2H_4—O—C_2H_4—$$

is a polyethylene glycol moiety having four polyethylene glycol subunits and a hydrophilic moiety having two polyethylene glycol subunits. Preferably, oligomers according to embodiments of the present invention comprise a polyalkylene glycol moiety and do not further comprise a hydrophilic moiety.

The oligomer may further comprise one or more lipophilic moieties as will be understood by those skilled in the art. The lipophilic moiety is preferably a saturated or unsaturated, linear or branched alkyl moiety or a saturated or unsaturated, linear or branched fatty acid moiety. When the lipophilic moiety is an alkyl moiety, it is preferably a linear, saturated or unsaturated alkyl moiety having 1 to 28 carbon atoms. More preferably, the alkyl moiety has 2 to 12 carbon atoms. When the lipophilic moiety is a fatty acid moiety, it is preferably a natural fatty acid moiety that is linear, saturated or unsaturated, having 2 to 18 carbon atoms. More preferably, the fatty acid moiety has 3 to 14 carbon atoms. Most preferably, the fatty acid moiety has at least 4, 5 or 6 carbon atoms.

The oligomer may further comprise one or more spacer moieties as will be understood by those skilled in the art. Spacer moieties may, for example, be used to separate a hydrophilic moiety from a lipophilic moiety, to separate a lipophilic moiety or hydrophilic moiety from the drug, to separate a first hydrophilic or lipophilic moiety from a second hydrophilic or lipophilic moiety, or to separate a hydrophilic moiety or lipophilic moiety from a linker moiety. Spacer moieties are preferably selected from the group consisting of sugar, cholesterol and glycerine moieties.

The oligomer may further comprise one or more linker moieties that are used to couple the oligomer with the drug as will be understood by those skilled in the art. Linker moieties are preferably selected from the group consisting of alkyl and fatty acid moieties.

The oligomer may further comprise one or more terminating moieties at the one or more ends of the oligomer which are not coupled to the drug. The terminating moiety is preferably an alkyl or alkoxy moiety, and is more preferably a lower alkyl or lower alkoxy moiety. Most preferably, the terminating moiety is methyl or methoxy. While the terminating moiety is preferably an alkyl or alkoxy moiety, it is to be understood that the terminating moiety may be various moieties as will be understood by those skilled in the art including, but not limited to, sugars, cholesterol, alcohols, and fatty acids.

The oligomer is preferably covalently coupled to the drug. In some embodiments, the drug is coupled to the oligomer utilizing a hydrolyzable bond (e.g., an ester or carbonate bond). A hydrolyzable coupling may provide a drug-oligomer conjugate that acts as a prodrug. In certain instances, for example where the drug-oligomer conjugate is inactive (i.e., the conjugate lacks the ability to affect the body through the drug's primary mechanism of action), a hydrolyzable coupling may provide for a time-release or controlled-release effect, administering the drug over a given time period as one or more oligomers are cleaved from their respective drug-oligomer conjugates to provide the active drug. In other embodiments, the drug is coupled to the oligomer utilizing a non-hydrolyzable bond (e.g., a carbamate, amide, or ether bond). Use of a non-hydrolyzable bond may be preferable when it is desirable to allow the drug-oligomer conjugate to circulate in the bloodstream for an extended period of time, preferably at least 2 hours.

While the oligomer is preferably covalently coupled to the drug, it is to be understood that the oligomer may be non-covalently coupled to the drug to form a non-covalently conjugated drug-oligomer complex. As will be understood by those skilled in the art, non-covalent couplings include, but are not limited to, hydrogen bonding, ionic bonding, Van der Waals bonding, and micellular or liposomal encapsulation. According to embodiments of the present invention, oligomers may be suitably constructed, modified and/or appropriately functionalized to impart the ability for non-covalent conjugation in a selected manner (e.g., to impart hydrogen bonding capability), as will be understood by those skilled in the art. According to other embodiments of present invention, oligomers may be derivatized with various compounds including, but not limited to, amino acids, oligopeptides, peptides, bile acids, bile acid derivatives, fatty acids, fatty acid derivatives, salicylic acids, salicylic acid derivatives, aminosalicylic acids, and aminosalicylic acid derivatives. The resulting oligomers can non-covalently couple (complex) with drug molecules, pharmaceutical

products, and/or pharmaceutical excipients. The resulting complexes preferably have balanced lipophilic and hydrophilic properties. According to still other embodiments of the present invention, oligomers may be derivatized with amine and/or alkyl amines. Under suitable acidic conditions, the resulting oligomers can form non-covalently conjugated complexes with drug molecules, pharmaceutical products and/or pharmaceutical excipients. The products resulting from such complexation preferably have balanced lipophilic and hydrophilic properties.

More than one oligomer (i.e., a plurality of oligomers) may be coupled to the drug. The oligomers in the plurality are preferably the same. However, it is to be understood that the oligomers in the plurality may be different from one another, or, alternatively, some of the oligomers in the plurality may be the same and some may be different. When a plurality of oligomers are coupled to the drug, it may be preferable to couple one or more of the oligomers to the drug with hydrolyzable bonds and couple one or more of the oligomers to the drug with non-hydrolyzable bonds. Alternatively, all of the bonds coupling the plurality of oligomers to the drug may be hydrolyzable, but have varying degrees of hydrolyzability such that, for example, one or more of the oligomers is rapidly removed from the drug by hydrolysis in the body and one or more of the oligomers is slowly removed from the drug by hydrolysis in the body.

The oligomer may be coupled to the drug at various nucleophilic residues of the drug including, but not limited to, nucleophilic hydroxyl functions and/or amino functions. When the drug is a polypeptide, a nucleophilic hydroxyl function may be found, for example, at serine and/or tyrosine residues, and a nucleophilic amino function may be found, for example, at histidine and/or lysine residues, and/or at the one or more N-termini of the polypeptide. When an oligomer is coupled to the one or more N-termini of the polypeptide, the coupling preferably forms a secondary amine. For example, when the drug is human insulin, the oligomer may be coupled to an amino functionality of the insulin including the amino functionality of Gly^{A1}, the amino functionality of Phe^{B1}, and the amino functionality of Lys^{B29}. When one oligomer is coupled to the human insulin, the oligomer is preferably coupled to the amino functionality of Lys^{B29}. When two oligomers are coupled to the human insulin, the oligomers are preferably coupled to the amino functionality of Phe^{B1} and the amino functionality of Lys^{B29}. While more than one oligomer may be coupled to the human insulin, a higher activity (improved glucose lowering ability) is observed for the mono-

conjugated human insulin. As another example, when the drug is salmon calcitonin, the oligomer may be coupled to an amino functionality of the salmon calcitonin, including the amino functionality of Lys^{11}, Lys^{18} and the N-terminus. While one or more oligomers may be coupled to the salmon calcitonin, a higher activity (improved glucose lowering ability) is observed for the di-conjugated salmon calcitonin where an oligomer is coupled to the amino functionality of Lys^{11} and an oligomer is coupled to the amino functionality of Lys^{18}. As yet another example, when the drug is human growth hormone, the oligomer may be coupled to an amino functionality of Phe^{1}, Lys^{38}, Lys^{41}, Lys^{70}, Lys^{115}, Lys^{140}, Lys^{145}, Lys^{158}, Lys^{168}, and/or Lys^{172}.

Mixtures of drug-oligomer conjugates having a molecular weight distribution with a standard deviation of less than about 22 Daltons may be synthesized by various methods. For example, a mixture of oligomers having a molecular weight distribution with a standard deviation of less than about 22 Daltons consisting of carboxylic acid and polyethylene glycol is synthesized by contacting a mixture of carboxylic acid having a molecular weight distribution with a standard deviation of less than about 22 Daltons with a mixture of polyethylene glycol having a molecular weight distribution with a standard deviation of less than about 22 Daltons under conditions sufficient to provide a mixture of oligomers having a molecular weight distribution with a standard deviation of less than about 22 Daltons. The oligomers of the mixture having a molecular weight distribution with a standard deviation of less than about 22 Daltons are then activated so that they are capable of reacting with a drug to provide a drug-oligomer conjugate. One embodiment of a synthesis route for providing a mixture of activated oligomers having a molecular weight distribution with a standard deviation of less than about 22 Daltons is illustrated in **Figure 3** and described in Examples 11-18 hereinbelow. Another embodiment of a synthesis route for providing a mixture of activated oligomers having a molecular weight distribution with a standard deviation of less than about 22 Daltons is illustrated in **Figure 4** and described in Examples 19-24 hereinbelow. Still another embodiment of a synthesis route for providing a mixture of activated oligomers having a molecular weight distribution with a standard deviation of less than about 22 Daltons is illustrated in **Figure 5** and described in Examples 25-29 hereinbelow. Yet another embodiment of a synthesis route for providing a mixture of activated oligomers having a molecular weight distribution with a standard deviation of less than about 22 Daltons is illustrated in **Figure 6** and described in Examples 30-31

hereinbelow. Another embodiment of a synthesis route for providing a mixture of activated oligomers having a molecular weight distribution with a standard deviation of less than about 22 Daltons is illustrated in **Figure** 7 and described in Examples 32-37 hereinbelow. Still another embodiment of a synthesis route for providing a mixture of activated oligomers having a molecular weight distribution with a standard deviation of less than about 22 Daltons is illustrated in **Figure 8** and described in Example 38 hereinbelow. Yet another embodiment of a synthesis route for providing a mixture of activated oligomers having a molecular weight distribution with a standard deviation of less than about 22 Daltons is illustrated in **Figure** 9 and described in Example 39 hereinbelow. Another embodiment of a synthesis route for providing a mixture of activated oligomers having a molecular weight distribution with a standard deviation of less than about 22 Daltons is illustrated in **Figure 10** and described in Example 40 hereinbelow.

The mixture of activated oligomers having a molecular weight distribution with a standard deviation of less than about 22 Daltons may be reacted with a mixture of drugs having a standard deviation of less than about 22 Daltons under conditions sufficient to provide a mixture of drug-oligomer conjugates, as described, for example, in Examples 41-120 hereinbelow. As will be understood by those skilled in the art, the reaction conditions (e.g., selected molar ratios, solvent mixtures and/or pH) may be controlled such that the mixture of drug-oligomer conjugates resulting from the reaction of the mixture of activated oligomers having a molecular weight distribution with a standard deviation of less than about 22 Daltons and the mixture of drugs having a molecular weight distribution with a standard deviation of less than about 22 Daltons is a mixture having a molecular weight distribution with a standard deviation of less than about 22 Daltons. For example, conjugation at the amino functionality of lysine may be suppressed by maintaining the pH of the reaction solution below the pK_a of lysine. Alternatively, the mixture of drug-oligomer conjugates may be separated and isolated utilizing, for example, HPLC to provide a mixture of drug-oligomer conjugates, for example mono-, di-, or tri-conjugates, having a molecular weight distribution with a standard deviation of less than about 22 Daltons. The degree of conjugation (e.g., whether the isolated molecule is a mono-, di-, or tri-conjugate) of a particular isolated conjugate may be determined and/or verified utilizing various techniques as will be understood by those skilled in the art including, but not limited to, mass spectroscopy. The particular conjugate structure (e.g., whether the oligomer is at Gly^{A1}, Phe^{B1}, or Lys^{B29} of a

human insulin monoconjugate) may be determined and/or verified utilizing various techniques as will be understood by those skilled in the art including, but not limited to, sequence analysis, peptide mapping, selective enzymatic cleavage, and/or endopeptidase cleavage.

As will be understood by those skilled in the art, one or more of the reaction sites on the drug may be blocked by, for example, reacting the drug with a suitable blocking reagent such as N-tert-butoxycarbonyl (t-BOC), or N-(9-fluorenylmethoxycarbonyl) (N-FMOC). This process may be preferred, for example, when the drug is a polypeptide and it is desired to form an unsaturated conjugate (i.e., a conjugate wherein not all nucleophilic residues are conjugated) having one or more oligomers at the one or more N-termini of the polypeptide. Following such blocking, the mixture of blocked drugs having a molecular weight distribution with a standard deviation of less than about 22 Daltons may be reacted with the mixture of activated oligomers having a molecular weight distribution with a standard deviation of less than about 22 Daltons to provide a mixture of drug-oligomer conjugates having oligomer(s) coupled to one or more nucleophilic residues and having blocking moieties coupled to other nucleophilic residues. After the conjugation reaction, the drug-oligomer conjugates may be de-blocked as will be understood by those skilled in the art. If necessary, the mixture of drug-oligomer conjugates may then be separated as described above to provide a mixture of drug-oligomer conjugates having a molecular weight distribution with a standard deviation of less than about 22 Daltons. Alternatively, the mixture of drug-oligomer conjugates may be separated prior to de-blocking.

Mixtures of drug-oligomer conjugates having a molecular weight distribution with a standard deviation of less than about 22 Daltons according to embodiments of the present invention preferably have improved properties when compared with those of conventional mixtures. For example, a mixture of drug-oligomer conjugates having a molecular weight distribution with a standard deviation of less than about 22 Daltons preferably has an *in vivo* activity that is greater than the *in vivo* activity of a polydispersed mixture of drug-oligomer conjugates having the same number average molecular weight as the mixture of drug-oligomer conjugates having a molecular weight distribution with a standard deviation of less than about 22 Daltons. As will be understood by those skilled in the art, the number average molecular weight of the mixture of drug-oligomer conjugates having a molecular weight distribution with a standard deviation of less than about 22 Daltons and the number average

weight of the polydispersed mixture may be measured by various methods including, but not limited to, size exclusion chromatography such as gel permeation chromatography as described, for example, in H.R. Allcock & F.W. Lampe, CONTEMPORARY POLYMER CHEMISTRY 394-402 (2d. ed., 1991).

As another example, a mixture of drug-oligomer conjugates having a molecular weight distribution with a standard deviation of less than about 22 Daltons preferably has an *in vitro* activity that is greater than the *in vitro* activity of a polydispersed mixture of drug-oligomer conjugates having the same number average molecular weight as the mixture of drug-oligomer conjugates having a molecular weight distribution with a standard deviation of less than about 22 Daltons. As will be understood by those skilled in the art, the number average molecular weight of the mixture of drug-oligomer conjugates having a molecular weight distribution with a standard deviation of less than about 22 Daltons and the number average weight of the polydispersed mixture may be measured by various methods including, but not limited to, size exclusion chromatography such as gel permeation chromatography.

The *in vitro* activity of a particular mixture may be measured by various methods, as will be understood by those skilled in the art. Preferably, the *in vitro* activity is measured using a Cytosensor® Microphysiometer commercially available from Molecular Devices Corporation of Sunnyvale, California. The microphysiometer monitors small changes in the rates of extracellular acidification in response to a drug being added to cultured cells in a transwell. This response is proportional to the activity of the molecule under study.

As still another example, a mixture of drug-oligomer conjugates having a molecular weight distribution with a standard deviation of less than about 22 Daltons preferably has an increased resistance to degradation by chymotrypsin when compared to the resistance to degradation by chymotrypsin of a polydispersed mixture of drug-oligomer conjugates having the same number average molecular weight as the mixture of drug-oligomer conjugates having a molecular weight distribution with a standard deviation of less than about 22 Daltons. As will be understood by those skilled in the art, the number average molecular weight of the mixture of drug-oligomer conjugates having a molecular weight distribution with a standard deviation of less than about 22 Daltons and the number average weight of the polydispersed mixture may be measured by various methods including, but not limited to, size exclusion chromatography.

As yet another example, a mixture of drug-oligomer conjugates having a molecular weight distribution with a standard deviation of less than about 22 Daltons preferably has an inter-subject variability that is less than the inter-subject variability of a polydispersed mixture of drug-oligomer conjugates having the same number average molecular weight as the mixture of drug-oligomer conjugates having a molecular weight distribution with a standard deviation of less than about 22 Daltons. As will be understood by those skilled in the art, the number average molecular weight of the mixture of drug-oligomer conjugates having a molecular weight distribution with a standard deviation of less than about 22 Daltons and the number average weight of the polydispersed mixture may be measured by various methods including, but not limited to, size exclusion chromatography. The inter-subject variability may be measured by various methods as will be understood by those skilled in the art. The inter-subject variability is preferably calculated as follows. The area under a dose response curve (AUC) (i.e., the area between the dose-response curve and a baseline value) is determined for each subject. The average AUC for all subjects is determined by summing the AUCs of each subject and dividing the sum by the number of subjects. The absolute value of the difference between the subject's AUC and the average AUC is then determined for each subject. The absolute values of the differences obtained are then summed to give a value that represents the inter-subject variability. Lower values represent lower inter-subject variabilities and higher values represent higher inter-subject variabilities.

Mixtures of drug-oligomer conjugates having a molecular weight distribution with a standard deviation of less than about 22 Daltons according to embodiments of the present invention preferably have two or more of the above-described improved properties. More preferably, mixtures of drug-oligomer conjugates having a molecular weight distribution with a standard deviation of less than about 22 Daltons according to embodiments of the present invention have three or more of the above-described improved properties. Most preferably, mixtures of drug-oligomer conjugates having a molecular weight distribution with a standard deviation of less than about 22 Daltons according to embodiments of the present invention have all four of the above-described improved properties.

According to yet other embodiments of the present invention, a mixture of conjugates is provided where each conjugate includes a drug coupled to an oligomer that comprises a

polyalkylene glycol moiety, and the mixture has a dispersity coefficient (DC) greater than 10,000 where

$$DC = \frac{\left(\sum_{i=1}^{n} N_i M_i\right)^2}{\sum_{i=1}^{n} N_i M_i^2 \sum_{i=1}^{n} N_i - \left(\sum_{i=1}^{n} N_i M_i\right)^2}$$

wherein:

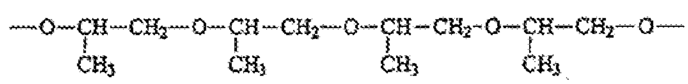
n is the number of different molecules in the sample;

N_i is the number of i^{th} molecules in the sample; and

M_i is the mass of the i^{th} molecule.

The mixture of conjugates preferably has a dispersity coefficient greater than 100,000. More preferably, the dispersity coefficient of the conjugate mixture is greater than 500,000 and, most preferably, the dispersity coefficient is greater than 10,000,000. The variables n, N_i, and M_i may be determined by various methods as will be understood by those skilled in the art, including, but not limited to, methods described below in Example 123.

The oligomer may be various oligomers comprising a polyalkylene glycol moiety as will be understood by those skilled in the art. Preferably, the polyalkylene glycol moiety has at least 2, 3, or 4 polyalkylene glycol subunits. More preferably, the polyalkylene glycol moiety has at least 5 or 6 polyalkylene glycol subunits. Most preferably, the polyalkylene glycol moiety of the oligomer has at least 7 polyalkylene glycol subunits. The polyalkylene glycol moiety of the oligomer is preferably a lower alkyl polyalkylene glycol moiety such as a polyethylene glycol moiety, a polypropylene glycol moiety, or a polybutylene glycol moiety. When the polyalkylene moiety is a polypropylene glycol moiety, the polypropylene glycol moiety preferably has a uniform structure. An exemplary polypropylene glycol moiety having a uniform structure is as follows:



This uniform polypropylene glycol structure may be described as having only one methyl substituted carbon atom adjacent each oxygen atom in the polypropylene glycol chain. Such uniform polypropylene glycol moieties may exhibit both lipophilic and hydrophilic characteristics and thus be useful in providing amphiphilic drug-oligomer conjugates without the use of lipophilic polymer moieties. Furthermore, coupling the secondary alcohol moiety

of the polypropylene glycol moiety with a drug may provide the drug (e.g., a polypeptide) with improved resistance to degradation caused by enzymes such as trypsin and chymotrypsin found, for example, in the gut.

Uniform polypropylene glycol according to embodiments of the present invention is preferably synthesized as illustrated in **Figures 11** through **13**, which will now be described. As illustrated in **Figure 11**, 1,2-propanediol **53** is reacted with a primary alcohol blocking reagent to provide a secondary alcohol extension monomer **54**. The primary alcohol blocking reagent may be various primary alcohol blocking reagents as will be understood by those skilled in the art including, but not limited to, silylchloride compounds such as t-butyldiphenylsilylchloride and t-butyldimethylsilylchloride, and esterification reagents such as Ac_2O. Preferably, the primary alcohol blocking reagent is a primary alcohol blocking reagent that is substantially non-reactive with secondary alcohols, such as t-butyldiphenylsilylchloride or t-butyldimethylsilylchloride. The secondary alcohol extension monomer (**54**) may be reacted with methanesulfonyl chloride ($MeSO_2Cl$) to provide a primary extension alcohol monomer mesylate **55**.

Alternatively, the secondary alcohol extension monomer **54** may be reacted with a secondary alcohol blocking reagent to provide compound **56**. The secondary alcohol blocking reagent may be various secondary alcohol blocking reagents as will be understood by those skilled in the art including, but not limited to, benzyl chloride. The compound **56** may be reacted with a B_1 de-blocking reagent to remove the blocking moiety B_1 and provide a primary alcohol extension monomer **57**. The B_1 de-blocking reagent may be selected from various de-blocking reagents as will be understood by one skilled in the art. When the primary alcohol has been blocked by forming an ester, the B_1 de-blocking reagent is a de-esterification reagent, such as a base (e.g., potassium carbonate). When the primary alcohol has been blocked using a silylchloride, the B_1 de-blocking reagent is preferably tetrabutylammonium fluoride (TBAF). The primary alcohol extension monomer **57** may be reacted with methane sulfonyl chloride to provide a secondary alcohol extension monomer mesylate **58**.

The primary alcohol extension monomer **54** and the secondary alcohol extension monomer **57** may be capped as follows. The secondary alcohol extension monomer **54** may be reacted with a capping reagent to provide a compound **59**. The capping reagent may be various capping reagents as will be understood by those skilled in the art including, but not

limited to, alkyl halides such as methyl chloride. The compound **59** may be reacted with a B_1 de-blocking agent as described above to provide a primary alcohol capping monomer **60**. The primary alcohol capping monomer **60** may be reacted with methane sulfonyl chloride to provide the secondary alcohol capping monomer mesylate **61**. The primary alcohol extension monomer **57** may be reacted with a capping reagent to provide a compound **62**. The capping reagent may be various capping reagents as described above. The compound 62 may be reacted with a B_2 de-blocking reagent to remove the blocking moiety B_2 and provide a secondary alcohol capping monomer **63**. The B_2 de-blocking reagent may be various de-blocking agents as will be understood by those skilled in the art including, but not limited to, H_2 in the presence of a palladium/activated carbon catalyst. The secondary alcohol capping monomer may be reacted with methanesulfonyl chloride to provide a primary alcohol capping monomer mesylate **64**. While the embodiments illustrated in **Figure 11** show the synthesis of capping monomers, it is to be understood that similar reactions may be performed to provide capping polymers.

In general, chain extensions may be effected by reacting a primary alcohol extension mono- or poly-mer such as the primary alcohol extension monomer **57** with a primary alcohol extension mono- or poly-mer mesylate such as the primary alcohol extension monomer mesylate **55** to provide various uniform polypropylene chains or by reacting a secondary alcohol extension mono- or poly-mer such as the secondary alcohol extension monomer **54** with a secondary alcohol extension mono-or poly-mer mesylate such as the secondary alcohol extension monomer mesylate **58**.

For example, in **Figure 13**, the primary alcohol extension monomer mesylate **55** is reacted with the primary alcohol extension monomer **57** to provide a dimer compound **65**. Alternatively, the secondary alcohol extension monomer mesylate **58** may be reacted with the secondary alcohol extension monomer **54** to provide the dimer compound **65**. The B_1 blocking moiety on the dimer compound **65** may be removed using a B_1 de-blocking reagent as described above to provide a primary alcohol extension dimer **66**. The primary alcohol extension dimer **66** may be reacted with methane sulfonyl chloride to provide a secondary alcohol extension dimer mesylate **67**. Alternatively, the B_2 blocking moiety on the dimer compound **65** may be removed using the B_2 de-blocking reagent as described above to provide a secondary alcohol extension dimer **69**. The secondary alcohol extension dimer **69**

may be reacted with methane sulfonyl chloride to provide a primary alcohol extension dimer mesylate **70**.

As will be understood by those skilled in the art, the chain extension process may be repeated to achieve various other chain lengths. For example, as illustrated in **Figure 13**, the primary alcohol extension dimer **66** may be reacted with the primary alcohol extension dimer mesylate **70** to provide a tetramer compound **72**. As further illustrated in **Figure 13**, a generic chain extension reaction scheme involves reacting the primary alcohol extension mono- or poly-mer **73** with the primary alcohol extension mono- or poly-mer mesylate **74** to provide the uniform polypropylene polymer **75**. The values of m and n may each range from 0 to 1000 or more. Preferably, m and n are each from 0 to 50. While the embodiments illustrated in **Figure 13** show primary alcohol extension mono- and/or poly-mers being reacted with primary alcohol extension mono- and/or poly-mer mesylates, it is to be understood that similar reactions may be carried out using secondary alcohol extension mono- and/or poly-mers and secondary alcohol extension mono- and/or poly-mer mesylates.

An end of a primary alcohol extension mono- or poly-mer or an end of a primary alcohol extension mono- or poly-mer mesylate may be reacted with a primary alcohol capping mono- or poly-mer mesylate or a primary alcohol capping mono- or poly-mer, respectively, to provide a capped uniform polypropylene chain. For example, as illustrated in **Figure 12**, the primary alcohol extension dimer mesylate **70** is reacted with the primary alcohol capping monomer **60** to provide the capped/blocked primary alcohol extension trimer **71**. As will be understood by those skilled in the art, the B_1 blocking moiety may be removed and the resulting capped primary alcohol extension trimer may be reacted with a primary alcohol extension mono- or poly-mer mesylate to extend the chain of the capped trimer **71**.

An end of a secondary alcohol extension mono-or poly-mer or an end of a secondary alcohol extension mono-or poly-mer mesylate may be reacted with a secondary alcohol capping mono-or poly-mer mesylate or a secondary alcohol capping mono- or poly-mer, respectively, to provide a capped uniform polypropylene chain. For example, as illustrated in **Figure 12**, the secondary alcohol extension dimer mesylate **67** is reacted with the secondary alcohol capping monomer **63** to provide the capped/blocked primary alcohol extension trimer **68**. The B_2 blocking moiety may be removed as described above and the resulting capped secondary alcohol extension trimer may be reacted with a secondary alcohol extension mer mesylate to extend the chain of the capped trimer **68**. While the syntheses illustrated in

Figure 12 show the reaction of a dimer with a capping monomer to provide a trimer, it is to be understood that the capping process may be performed at any point in the synthesis of a uniform polypropylene glycol moiety, or, alternatively, uniform polypropylene glycol moieties may be provided that are not capped. While the embodiments illustrated in Figure 12 show the capping of a polybutylene oligomer by synthesis with a capping monomer, it is to be understood that polybutylene oligomers of the present invention may be capped directly (i.e., without the addition of a capping monomer) using a capping reagent as described above in Figure 11.

Uniform polypropylene glycol moieties according to embodiments of the present invention may be coupled to a drug, a lipophilic moiety such as a carboxylic acid, and/or various other moieties by various methods as will be understood by those skilled in the art including, but not limited to, those described herein with respect to polyethylene glycol moieties.

The oligomer may comprise one or more other moieties as will be understood by those skilled in the art including, but not limited to, hydrophilic moieties, lipophilic moieties, spacer moieties, linker moieties, and terminating moieties. The various moieties in the oligomer are covalently coupled to one another by either hydrolyzable or non-hydrolyzable bonds.

The oligomer may further comprise one or more hydrophilic moieties including, but not limited to, sugars, polyalkylene glycols, and polyamine/PEG copolymers. Adjacent polyalkylene glycol moieties will be considered to be the same moiety if they are coupled by an ether bond and have the same alkyl structure. For example, the moiety

$$-O-C_2H_4-O-C_2H_4-O-C_2H_4-O-C_2H_4-O-C_2H_4-O-C_2H_4-$$

is a single polyethylene glycol moiety having six polyethylene glycol subunits. Adjacent polyalkylene glycol moieties will be considered to be different moieties if they are coupled by a bond other than an ether bond or if they have different alkyl structures. For example, the moiety

$$-O-C_2H_4-O-C_2H_4-O-C_2H_4-O-C_2H_4-\overset{\displaystyle O}{\overset{\|}{C}}-O-C_2H_4-O-C_2H_4-$$

is a polyethylene glycol moiety having four polyethylene glycol subunits and a hydrophilic moiety having two polyethylene glycol subunits. Preferably, oligomers according to

embodiments of the present invention comprise a polyalkylene glycol moiety and do not further comprise a hydrophilic moiety.

The oligomer may further comprise one or more lipophilic moieties as will be understood by those skilled in the art. The lipophilic moiety is preferably a saturated or unsaturated, linear or branched alkyl moiety or a saturated or unsaturated, linear or branched fatty acid moiety. When the lipophilic moiety is an alkyl moiety, it is preferably a linear, saturated or unsaturated alkyl moiety having 1 to 28 carbon atoms. More preferably, the alkyl moiety has 2 to 12 carbon atoms. When the lipophilic moiety is a fatty acid moiety, it is preferably a natural fatty acid moiety that is linear, saturated or unsaturated, having 2 to 18 carbon atoms. More preferably, the fatty acid moiety has 3 to 14 carbon atoms. Most preferably, the fatty acid moiety has at least 4, 5 or 6 carbon atoms.

The oligomer may further comprise one or more spacer moieties as will be understood by those skilled in the art. Spacer moieties may, for example, be used to separate a hydrophilic moiety from a lipophilic moiety, to separate a lipophilic moiety or hydrophilic moiety from the drug, to separate a first hydrophilic or lipophilic moiety from a second hydrophilic or lipophilic moiety, or to separate a hydrophilic moiety or lipophilic moiety from a linker moiety. Spacer moieties are preferably selected from the group consisting of sugar, cholesterol and glycerine moieties.

The oligomer may further comprise one or more linker moieties that are used to couple the oligomer with the drug as will be understood by those skilled in the art. Linker moieties are preferably selected from the group consisting of alkyl and fatty acid moieties.

The oligomer may further comprise one or more terminating moieties at the one or more ends of the oligomer which are not coupled to the drug. The terminating moiety is preferably an alkyl or alkoxy moiety, and is more preferably a lower alkyl or lower alkoxy moiety. Most preferably, the terminating moiety is methyl or methoxy. While the terminating moiety is preferably an alkyl or alkoxy moiety, it is to be understood that the terminating moiety may be various moieties as will be understood by those skilled in the art including, but not limited to, sugars, cholesterol, alcohols, and fatty acids.

The oligomer is preferably covalently coupled to the drug. In some embodiments, the drug is coupled to the oligomer utilizing a hydrolyzable bond (e.g., an ester or carbonate bond). A hydrolyzable coupling may provide a drug-oligomer conjugate that acts as a prodrug. In certain instances, for example where the drug-oligomer conjugate is inactive

(i.e., the conjugate lacks the ability to affect the body through the drug's primary mechanism of action), a hydrolyzable coupling may provide for a time-release or controlled-release effect, administering the drug over a given time period as one or more oligomers are cleaved from their respective drug-oligomer conjugates to provide the active drug. In other embodiments, the drug is coupled to the oligomer utilizing a non-hydrolyzable bond (e.g., a carbamate, amide, or ether bond). Use of a non-hydrolyzable bond may be preferable when it is desirable to allow the drug-oligomer conjugate to circulate in the bloodstream for an extended period of time, preferably at least 2 hours.

While the oligomer is preferably covalently coupled to the drug, it is to be understood that the oligomer may be non-covalently coupled to the drug to form a non-covalently conjugated drug-oligomer complex. As will be understood by those skilled in the art, non-covalent couplings include, but are not limited to, hydrogen bonding, ionic bonding, Van der Waals bonding, and micellular or liposomal encapsulation. According to embodiments of the present invention, oligomers may be suitably constructed, modified and/or appropriately functionalized to impart the ability for non-covalent conjugation in a selected manner (e.g., to impart hydrogen bonding capability), as will be understood by those skilled in the art. According to other embodiments of present invention, oligomers may be derivatized with various compounds including, but not limited to, amino acids, oligopeptides, peptides, bile acids, bile acid derivatives, fatty acids, fatty acid derivatives, salicylic acids, salicylic acid derivatives, aminosalicylic acids, and aminosalicylic acid derivatives. The resulting oligomers can non-covalently couple (complex) with drug molecules, pharmaceutical products, and/or pharmaceutical excipients. The resulting complexes preferably have balanced lipophilic and hydrophilic properties. According to still other embodiments of the present invention, oligomers may be derivatized with amine and/or alkyl amines. Under suitable acidic conditions, the resulting oligomers can form non-covalently conjugated complexes with drug molecules, pharmaceutical products and/or pharmaceutical excipients. The products resulting from such complexation preferably have balanced lipophilic and hydrophilic properties.

More than one oligomer (i.e., a plurality of oligomers) may be coupled to the drug. The oligomers in the plurality are preferably the same. However, it is to be understood that the oligomers in the plurality may be different from one another, or, alternatively, some of the oligomers in the plurality may be the same and some may be different. When a plurality of

oligomers are coupled to the drug, it may be preferable to couple one or more of the oligomers to the drug with hydrolyzable bonds and couple one or more of the oligomers to the drug with non-hydrolyzable bonds. Alternatively, all of the bonds coupling the plurality of oligomers to the drug may be hydrolyzable, but have varying degrees of hydrolyzability such that, for example, one or more of the oligomers is rapidly removed from the drug by hydrolysis in the body and one or more of the oligomers is slowly removed from the drug by hydrolysis in the body.

The oligomer may be coupled to the drug at various nucleophilic residues of the drug including, but not limited to, nucleophilic hydroxyl functions and/or amino functions. When the drug is a polypeptide, a nucleophilic hydroxyl function may be found, for example, at serine and/or tyrosine residues, and a nucleophilic amino function may be found, for example, at histidine and/or lysine residues, and/or at the one or more N-termini of the polypeptide. When an oligomer is coupled to the one or more N-termini of the polypeptide, the coupling preferably forms a secondary amine. For example, when the drug is human insulin, the oligomer may be coupled to an amino functionality of the insulin including the amino functionality of Gly^{A1}, the amino functionality of Phe^{B1}, and the amino functionality of Lys^{B29}. When one oligomer is coupled to the human insulin, the oligomer is preferably coupled to the amino functionality of Lys^{B29}. When two oligomers are coupled to the human insulin, the oligomers are preferably coupled to the amino functionality of Phe^{B1} and the amino functionality of Lys^{B29}. While more than one oligomer may be coupled to the human insulin, a higher activity (improved glucose lowering ability) is observed for the mono-conjugated human insulin. As another example, when the drug is salmon calcitonin, the oligomer may be coupled to an amino functionality of the salmon calcitonin, including the amino functionality of Lys^{11}, Lys^{18} and the N-terminus. While one or more oligomers may be coupled to the salmon calcitonin, a higher activity (improved glucose lowering ability) is observed for the di-conjugated salmon calcitonin where an oligomer is coupled to the amino functionality of Lys^{11} and an oligomer is coupled to the amino functionality of Lys^{18}. As yet another example, when the drug is human growth hormone, the oligomer may be coupled to an amino functionality of Phe^{1}, Lys^{38}, Lys^{41}, Lys^{70}, Lys^{115}, Lys^{140}, Lys^{145}, Lys^{158}, Lys^{168}, and/or Lys^{172}.

Mixtures of drug-oligomer conjugates having a dispersity coefficient greater than 10,000 may be synthesized by various methods. For example, a mixture of oligomers having

a dispersity coefficient greater than 10,000 consisting of carboxylic acid and polyethylene glycol is synthesized by contacting a mixture of carboxylic acid having a dispersity coefficient greater than 10,000 with a mixture of polyethylene glycol having a dispersity coefficient greater than 10,000 under conditions sufficient to provide a mixture of oligomers having a dispersity coefficient greater than 10,000. The oligomers of the mixture having a dispersity coefficient greater than 10,000 are then activated so that they are capable of reacting with a drug to provide a drug-oligomer conjugate. One embodiment of a synthesis route for providing a mixture of activated oligomers having a dispersity coefficient greater than 10,000 is illustrated in **Figure** 3 and described in Examples 11-18 hereinbelow. Another embodiment of a synthesis route for providing a mixture of activated oligomers having a dispersity coefficient greater than 10,000 is illustrated in **Figure 4** and described in Examples 19-24 hereinbelow. Still another embodiment of a synthesis route for providing a mixture of activated oligomers having a dispersity coefficient greater than 10,000 is illustrated in **Figure 5** and described in Examples 25-29 hereinbelow. Yet another embodiment of a synthesis route for providing a mixture of activated oligomers having a dispersity coefficient greater than 10,000 is illustrated in **Figure 6** and described in Examples 30-31 hereinbelow. Another embodiment of a synthesis route for providing a mixture of activated oligomers having a dispersity coefficient greater than 10,000 is illustrated in **Figure 7** and described in Examples 32-37 hereinbelow. Still another embodiment of a synthesis route for providing a mixture of activated oligomers having a dispersity coefficient greater than 10,000 is illustrated in **Figure 8** and described in Example 38 hereinbelow. Yet another embodiment of a synthesis route for providing a mixture of activated oligomers having a dispersity coefficient greater than 10,000 is illustrated in **Figure 9** and described in Example 39 hereinbelow. Another embodiment of a synthesis route for providing a mixture of activated oligomers having a dispersity coefficient greater than 10,000 is illustrated in **Figure 10** and described in Example 40 hereinbelow.

The mixture of activated oligomers having a dispersity coefficient greater than 10,000 is reacted with a mixture of drugs having a dispersity coefficient greater than 10,000 under conditions sufficient to provide a mixture of drug-oligomer conjugates, as described, for example, in Examples 41-120 hereinbelow. As will be understood by those skilled in the art, the reaction conditions (e.g., selected molar ratios, solvent mixtures and/or pH) may be controlled such that the mixture of drug-oligomer conjugates resulting from the reaction of

the mixture of activated oligomers having a dispersity coefficient greater than 10,000 and the mixture of drugs having a dispersity coefficient greater than 10,000 is a mixture having a dispersity coefficient greater than 10,000. For example, conjugation at the amino functionality of lysine may be suppressed by maintaining the pH of the reaction solution below the pK_a of lysine. Alternatively, the mixture of drug-oligomer conjugates may be separated and isolated utilizing, for example, HPLC to provide a mixture of drug-oligomer conjugates, for example mono-, di-, or tri-conjugates, having a dispersity coefficient greater than 10,000. The degree of conjugation (e.g., whether the isolated molecule is a mono-, di-, or tri-conjugate) of a particular isolated conjugate may be determined and/or verified utilizing various techniques as will be understood by those skilled in the art including, but not limited to, mass spectroscopy. The particular conjugate structure (e.g., whether the oligomer is at Gly^{A1} Phe^{B1}, or Lys^{B29} of a human insulin monoconjugate) may be determined and/or verified utilizing various techniques as will be understood by those skilled in the art including, but not limited to, sequence analysis, peptide mapping, selective enzymatic cleavage, and/or endopeptidase cleavage.

As will be understood by those skilled in the art, one or more of the reaction sites on the drug may be blocked by, for example, reacting the drug with a suitable blocking reagent such as N-tert-butoxycarbonyl (t-BOC), or N-(9-fluorenylmethoxycarbonyl) (N-FMOC). This process may be preferred, for example, when the drug is a polypeptide and it is desired to form an unsaturated conjugate (i.e., a conjugate wherein not all nucleophilic residues are conjugated) having an oligomer at the one or more N-termini of the polypeptide. Following such blocking, the mixture of blocked drugs having a dispersity coefficient greater than 10,000 may be reacted with the mixture of activated oligomers having a dispersity coefficient greater than 10,000 to provide a mixture of drug-oligomer conjugates having oligomer(s) coupled to one or more nucleophilic residues and having blocking moieties coupled to other nucleophilic residues. After the conjugation reaction, the drug-oligomer conjugates may be de-blocked as will be understood by those skilled in the art. If necessary, the mixture of drug-oligomer conjugates may then be separated as described above to provide a mixture of drug-oligomer conjugates having a dispersity coefficient greater than 10,000. Alternatively, the mixture of drug-oligomer conjugates may be separated prior to de-blocking.

Mixtures of drug-oligomer conjugates having a dispersity coefficient greater than 10,000 according to embodiments of the present invention preferably have improved

properties when compared with those of conventional mixtures. For example, a mixture of drug-oligomer conjugates having a dispersity coefficient greater than 10,000 preferably has an *in vivo* activity that is greater than the *in vivo* activity of a polydispersed mixture of drug-oligomer conjugates having the same number average molecular weight as the mixture of drug-oligomer conjugates having a dispersity coefficient greater than 10,000. As will be understood by those skilled in the art, the number average molecular weight of the mixture of drug-oligomer conjugates having a dispersity coefficient greater than 10,000 and the number average molecular weight of the polydispersed mixture may be measured by various methods including, but not limited to, size exclusion chromatography such as gel permeation chromatography as described, for example, in H.R. Allcock & F.W. Lampe, CONTEMPORARY POLYMER CHEMISTRY 394-402 (2d. ed., 1991).

As another example, a mixture of drug-oligomer conjugates having a dispersity coefficient greater than 10,000 preferably has an *in vitro* activity that is greater than the *in vitro* activity of a polydispersed mixture of drug-oligomer conjugates having the same number average molecular weight as the mixture of drug-oligomer conjugates having a dispersity coefficient greater than 10,000. As will be understood by those skilled in the art, the number average molecular weight of the mixture of drug-oligomer conjugates having a dispersity coefficient greater than 10,000 and the number average weight of the polydispersed mixture may be measured by various methods including, but not limited to, size exclusion chromatography.

The *in vitro* activity of a particular mixture may be measured by various methods, as will be understood by those skilled in the art. Preferably, the *in vitro* activity is measured using a Cytosensor® Microphysiometer commercially available from Molecular Devices Corporation of Sunnyvale, California. The microphysiometer monitors small changes in the rates of extracellular acidification in response to a drug being added to cultured cells in a transwell. This response is proportional to the activity of the molecule under study.

As still another example, a mixture of drug-oligomer conjugates having a dispersity coefficient greater than 10,000 preferably has an increased resistance to degradation by chymotrypsin when compared to the resistance to degradation by chymotrypsin of a polydispersed mixture of drug-oligomer conjugates having the same number average molecular weight as the mixture of drug-oligomer conjugates having a dispersity coefficient greater than 10,000. As will be understood by those skilled in the art, the number average

molecular weight of the mixture of drug-oligomer conjugates having a dispersity coefficient greater than 10,000 and the number average weight of the polydispersed mixture may be measured by various methods including, but not limited to, size exclusion chromatography.

As yet another example, a mixture of drug-oligomer conjugates having a dispersity coefficient greater than 10,000 preferably has an inter-subject variability that is less than the inter-subject variability of a polydispersed mixture of drug-oligomer conjugates having the same number average molecular weight as the mixture of drug-oligomer conjugates having a dispersity coefficient greater than 10,000. As will be understood by those skilled in the art, the number average molecular weight of the mixture of drug-oligomer conjugates having a dispersity coefficient greater than 10,000 and the number average weight of the polydispersed mixture may be measured by various methods including, but not limited to, size exclusion chromatography. The inter-subject variability may be measured by various methods as will be understood by those skilled in the art. The inter-subject variability is preferably calculated as follows. The area under a dose response curve (AUC) (i.e., the area between the dose-response curve and a baseline value) is determined for each subject. The average AUC for all subjects is determined by summing the AUCs of each subject and dividing the sum by the number of subjects. The absolute value of the difference between the subject's AUC and the average AUC is then determined for each subject. The absolute values of the differences obtained are then summed to give a value that represents the inter-subject variability. Lower values represent lower inter-subject variabilities and higher values represent higher inter-subject variabilities.

Mixtures of drug-oligomer conjugates having a dispersity coefficient greater than 10,000 according to embodiments of the present invention preferably have two or more of the above-described improved properties. More preferably, mixtures of drug-oligomer conjugates having a dispersity coefficient greater than 10,000 according to embodiments of the present invention have three or more of the above-described improved properties. Most preferably, mixtures of drug-oligomer conjugates having a dispersity coefficient greater than 10,000 according to embodiments of the present invention have all four of the above-described improved properties.

According to other embodiments of the present invention, a mixture of conjugates in which each conjugate includes a drug coupled to an oligomer and has the same number of polyalkylene glycol subunits is provided.

The oligomer may be various oligomers comprising a polyalkylene glycol moiety as will be understood by those skilled in the art. Preferably, the polyalkylene glycol moiety has at least 2, 3, or 4 polyalkylene glycol subunits. More preferably, the polyalkylene glycol moiety has at least 5 or 6 polyalkylene glycol subunits. Most preferably, the polyalkylene glycol moiety of the oligomer has at least 7 polyalkylene glycol subunits. The polyalkylene glycol moiety of the oligomer is preferably a lower alkyl polyalkylene glycol moiety such as a polyethylene glycol moiety, a polypropylene glycol moiety, or a polybutylene glycol moiety. When the polyalkylene moiety is a polypropylene glycol moiety, the polypropylene glycol moiety preferably has a uniform structure. An exemplary polypropylene glycol moiety having a uniform structure is as follows:

$$-O-\underset{\substack{|\\ CH_3}}{CH}-CH_2-O-\underset{\substack{|\\ CH_3}}{CH}-CH_2-O-\underset{\substack{|\\ CH_3}}{CH}-CH_2-O-\underset{\substack{|\\ CH_3}}{CH}-CH_2-O-$$

This uniform polypropylene glycol structure may be described as having only one methyl substituted carbon atom adjacent each oxygen atom in the polypropylene glycol chain. Such uniform polypropylene glycol moieties may exhibit both lipophilic and hydrophilic characteristics and thus be useful in providing amphiphilic drug-oligomer conjugates without the use of lipophilic polymer moieties. Furthermore, coupling the secondary alcohol moiety of the polypropylene glycol moiety with a drug may provide the drug (e.g., a polypeptide) with improved resistance to degradation caused by enzymes such as trypsin and chymotrypsin found, for example, in the gut.

Uniform polypropylene glycol according to embodiments of the present invention is preferably synthesized as illustrated in **Figures** 11 through 13, which will now be described. As illustrated in **Figure 11**, 1,2-propanediol **53** is reacted with a primary alcohol blocking reagent to provide a secondary alcohol extension monomer **54**. The primary alcohol blocking reagent may be various primary alcohol blocking reagents as will be understood by those skilled in the art including, but not limited to, silylchloride compounds such as t-butyldiphenylsilylchloride and t-butyldimethylsilylchloride, and esterification reagents such as Ac_2O. Preferably, the primary alcohol blocking reagent is a primary alcohol blocking reagent that is substantially non-reactive with secondary alcohols, such as t-butyldiphenylsilylchloride or t-butyldimethylsilylchloride. The secondary alcohol extension monomer (**54**) may be reacted with methanesulfonyl chloride ($MeSO_2Cl$) to provide a primary extension alcohol monomer mesylate **55**.

Alternatively, the secondary alcohol extension monomer 54 may be reacted with a secondary alcohol blocking reagent to provide compound 56. The secondary alcohol blocking reagent may be various secondary alcohol blocking reagents as will be understood by those skilled in the art including, but not limited to, benzyl chloride. The compound 56 may be reacted with a B_1 de-blocking reagent to remove the blocking moiety B_1 and provide a primary alcohol extension monomer 57. The B_1 de-blocking reagent may be selected from various de-blocking reagents as will be understood by one skilled in the art. When the primary alcohol has been blocked by forming an ester, the B_1 de-blocking reagent is a de-esterification reagent, such as a base (e.g., potassium carbonate). When the primary alcohol has been blocked using a silylchloride, the B_1 de-blocking reagent is preferably tetrabutylammonium fluoride (TBAF). The primary alcohol extension monomer 57 may be reacted with methane sulfonyl chloride to provide a secondary alcohol extension monomer mesylate 58.

The primary alcohol extension monomer 54 and the secondary alcohol extension monomer 57 may be capped as follows. The secondary alcohol extension monomer 54 may be reacted with a capping reagent to provide a compound 59. The capping reagent may be various capping reagents as will be understood by those skilled in the art including, but not limited to, alkyl halides such as methyl chloride. The compound 59 may be reacted with a B_1 de-blocking agent as described above to provide a primary alcohol capping monomer 60. The primary alcohol capping monomer 60 may be reacted with methane sulfonyl chloride to provide the secondary alcohol capping monomer mesylate 61. The primary alcohol extension monomer 57 may be reacted with a capping reagent to provide a compound 62. The capping reagent may be various capping reagents as described above. The compound 62 may be reacted with a B_2 de-blocking reagent to remove the blocking moiety B_2 and provide a secondary alcohol capping monomer 63. The B_2 de-blocking reagent may be various de-blocking agents as will be understood by those skilled in the art including, but not limited to, H_2 in the presence of a palladium/activated carbon catalyst. The secondary alcohol capping monomer may be reacted with methanesulfonyl chloride to provide a primary alcohol capping monomer mesylate 64. While the embodiments illustrated in **Figure 11** show the synthesis of capping monomers, it is to be understood that similar reactions may be performed to provide capping polymers.

In general, chain extensions may be effected by reacting a primary alcohol extension mono- or poly-mer such as the primary alcohol extension monomer **57** with a primary alcohol extension mono- or poly-mer mesylate such as the primary alcohol extension monomer mesylate **55** to provide various uniform polypropylene chains or by reacting a secondary alcohol extension mono- or poly-mer such as the secondary alcohol extension monomer **54** with a secondary alcohol extension mono-or poly-mer mesylate such as the secondary alcohol extension monomer mesylate **58**.

For example, in **Figure 13**, the primary alcohol extension monomer mesylate **55** is reacted with the primary alcohol extension monomer **57** to provide a dimer compound **65**. Alternatively, the secondary alcohol extension monomer mesylate **58** may be reacted with the secondary alcohol extension monomer **54** to provide the dimer compound **65**. The B_1 blocking moiety on the dimer compound **65** may be removed using a B_1 de-blocking reagent as described above to provide a primary alcohol extension dimer **66**. The primary alcohol extension dimer **66** may be reacted with methane sulfonyl chloride to provide a secondary alcohol extension dimer mesylate **67**. Alternatively, the B_2 blocking moiety on the dimer compound **65** may be removed using the B_2 de-blocking reagent as described above to provide a secondary alcohol extension dimer **69**. The secondary alcohol extension dimer **69** may be reacted with methane sulfonyl chloride to provide a primary alcohol extension dimer mesylate **70**.

As will be understood by those skilled in the art, the chain extension process may be repeated to achieve various other chain lengths. For example, as illustrated in **Figure 13**, the primary alcohol extension dimer **66** may be reacted with the primary alcohol extension dimer mesylate **70** to provide a tetramer compound **72**. As further illustrated in **Figure 13**, a generic chain extension reaction scheme involves reacting the primary alcohol extension mono- or poly-mer 73 with the primary alcohol extension mono- or poly-mer mesylate 74 to provide the uniform polypropylene polymer 75. The values of m and n may each range from 0 to 1000 or more. Preferably, m and n are each from 0 to 50. While the embodiments illustrated in **Figure 13** show primary alcohol extension mono- and/or poly-mers being reacted with primary alcohol extension mono- and/or poly-mer mesylates, it is to be understood that similar reactions may be carried out using secondary alcohol extension mono- and/or poly-mers and secondary alcohol extension mono- and/or poly-mer mesylates.

An end of a primary alcohol extension mono- or poly-mer or an end of a primary alcohol extension mono- or poly-mer mesylate may be reacted with a primary alcohol capping mono- or poly-mer mesylate or a primary alcohol capping mono- or poly-mer, respectively, to provide a capped uniform polypropylene chain. For example, as illustrated in **Figure 12**, the primary alcohol extension dimer mesylate **70** is reacted with the primary alcohol capping monomer **60** to provide the capped/blocked primary alcohol extension trimer **71**. As will be understood by those skilled in the art, the B_1 blocking moiety may be removed and the resulting capped primary alcohol extension trimer may be reacted with a primary alcohol extension mono- or poly-mer mesylate to extend the chain of the capped trimer **71**.

An end of a secondary alcohol extension mono-or poly-mer or an end of a secondary alcohol extension mono-or poly-mer mesylate may be reacted with a secondary alcohol capping mono-or poly-mer mesylate or a secondary alcohol capping mono- or poly-mer, respectively, to provide a capped uniform polypropylene chain. For example, as illustrated in **Figure 12**, the secondary alcohol extension dimer mesylate **67** is reacted with the secondary alcohol capping monomer **63** to provide the capped/blocked primary alcohol extension trimer **68**. The B_2 blocking moiety may be removed as described above and the resulting capped secondary alcohol extension trimer may be reacted with a secondary alcohol extension mer mesylate to extend the chain of the capped trimer **68**. While the syntheses illustrated in **Figure 12** show the reaction of a dimer with a capping monomer to provide a trimer, it is to be understood that the capping process may be performed at any point in the synthesis of a uniform polypropylene glycol moiety, or, alternatively, uniform polypropylene glycol moieties may be provided that are not capped. While the embodiments illustrated in **Figure 12** show the capping of a polybutylene oligomer by synthesis with a capping monomer, it is to be understood that polybutylene oligomers of the present invention may be capped directly (i.e., without the addition of a capping monomer) using a capping reagent as described above in **Figure 11**.

Uniform polypropylene glycol moieties according to embodiments of the present invention may be coupled to a drug, a lipophilic moiety such as a carboxylic acid, and/or various other moieties by various methods as will be understood by those skilled in the art including, but not limited to, those described herein with respect to polyethylene glycol moieties.

The oligomer may comprise one or more other moieties as will be understood by those skilled in the art including, but not limited to, hydrophilic moieties, lipophilic moieties, spacer moieties, linker moieties, and terminating moieties. The various moieties in the oligomer are covalently coupled to one another by either hydrolyzable or non-hydrolyzable bonds.

The oligomer may further comprise one or more hydrophilic moieties including, but not limited to, sugars, polyalkylene glycols, and polyamine/PEG copolymers. Adjacent polyalkylene glycol moieties will be considered to be the same moiety if they are coupled by an ether bond and have the same alkyl structure. For example, the moiety

$$—O—C_2H_4—O—C_2H_4—O—C_2H_4—O—C_2H_4—O—C_2H_4—O—C_2H_4—$$

is a single polyethylene glycol moiety having six polyethylene glycol subunits. Adjacent polyalkylene glycol moieties will be considered to be different moieties if they are coupled by a bond other than an ether bond or if they have different alkyl structures. For example, the moiety

$$—O—C_2H_4—O—C_2H_4—O—C_2H_4—O—C_2H_4—\overset{\overset{\displaystyle O}{\|}}{C}—O—C_2H_4—O—C_2H_4—$$

is a polyethylene glycol moiety having four polyethylene glycol subunits and a hydrophilic moiety having two polyethylene glycol subunits. Preferably, oligomers according to embodiments of the present invention comprise a polyalkylene glycol moiety and do not further comprise a hydrophilic moiety.

The oligomer may further comprise one or more lipophilic moieties as will be understood by those skilled in the art. The lipophilic moiety is preferably a saturated or unsaturated, linear or branched alkyl moiety or a saturated or unsaturated, linear or branched fatty acid moiety. When the lipophilic moiety is an alkyl moiety, it is preferably a linear, saturated or unsaturated alkyl moiety having 1 to 28 carbon atoms. More preferably, the alkyl moiety has 2 to 12 carbon atoms. When the lipophilic moiety is a fatty acid moiety, it is preferably a natural fatty acid moiety that is linear, saturated or unsaturated, having 2 to 18 carbon atoms. More preferably, the fatty acid moiety has 3 to 14 carbon atoms. Most preferably, the fatty acid moiety has at least 4, 5 or 6 carbon atoms.

The oligomer may further comprise one or more spacer moieties as will be understood by those skilled in the art. Spacer moieties may, for example, be used to separate a hydrophilic moiety from a lipophilic moiety, to separate a lipophilic moiety or hydrophilic

moiety from the drug, to separate a first hydrophilic or lipophilic moiety from a second hydrophilic or lipophilic moiety, or to separate a hydrophilic moiety or lipophilic moiety from a linker moiety. Spacer moieties are preferably selected from the group consisting of sugar, cholesterol and glycerine moieties.

The oligomer may further comprise one or more linker moieties that are used to couple the oligomer with the drug as will be understood by those skilled in the art. Linker moieties are preferably selected from the group consisting of alkyl and fatty acid moieties.

The oligomer may further comprise one or more terminating moieties at the one or more ends of the oligomer which are not coupled to the drug. The terminating moiety is preferably an alkyl or alkoxy moiety, and is more preferably a lower alkyl or lower alkoxy moiety. Most preferably, the terminating moiety is methyl or methoxy. While the terminating moiety is preferably an alkyl or alkoxy moiety, it is to be understood that the terminating moiety may be various moieties as will be understood by those skilled in the art including, but not limited to, sugars, cholesterol, alcohols, and fatty acids.

The oligomer is preferably covalently coupled to the drug. In some embodiments, the drug is coupled to the oligomer utilizing a hydrolyzable bond (e.g., an ester or carbonate bond). A hydrolyzable coupling may provide a drug-oligomer conjugate that acts as a prodrug. In certain instances, for example where the drug-oligomer conjugate is inactive (i.e., the conjugate lacks the ability to affect the body through the drug's primary mechanism of action), a hydrolyzable coupling may provide for a time-release or controlled-release effect, administering the drug over a given time period as one or more oligomers are cleaved from their respective drug-oligomer conjugates to provide the active drug. In other embodiments, the drug is coupled to the oligomer utilizing a non-hydrolyzable bond (e.g., a carbamate, amide, or ether bond). Use of a non-hydrolyzable bond may be preferable when it is desirable to allow the drug-oligomer conjugate to circulate in the bloodstream for an extended period of time, preferably at least 2 hours.

While the oligomer is preferably covalently coupled to the drug, it is to be understood that the oligomer may be non-covalently coupled to the drug to form a non-covalently conjugated drug-oligomer complex. As will be understood by those skilled in the art, non-covalent couplings include, but are not limited to, hydrogen bonding, ionic bonding, Van der Waals bonding, and micellular or liposomal encapsulation. According to embodiments of the present invention, oligomers may be suitably constructed, modified and/or appropriately

functionalized to impart the ability for non-covalent conjugation in a selected manner (e.g., to impart hydrogen bonding capability), as will be understood by those skilled in the art. According to other embodiments of present invention, oligomers may be derivatized with various compounds including, but not limited to, amino acids, oligopeptides, peptides, bile acids, bile acid derivatives, fatty acids, fatty acid derivatives, salicylic acids, salicylic acid derivatives, aminosalicylic acids, and aminosalicylic acid derivatives. The resulting oligomers can non-covalently couple (complex) with drug molecules, pharmaceutical products, and/or pharmaceutical excipients. The resulting complexes preferably have balanced lipophilic and hydrophilic properties. According to still other embodiments of the present invention, oligomers may be derivatized with amine and/or alkyl amines. Under suitable acidic conditions, the resulting oligomers can form non-covalently conjugated complexes with drug molecules, pharmaceutical products and/or pharmaceutical excipients. The products resulting from such complexation preferably have balanced lipophilic and hydrophilic properties.

More than one oligomer (i.e., a plurality of oligomers) may be coupled to the drug. The oligomers in the plurality are preferably the same. However, it is to be understood that the oligomers in the plurality may be different from one another, or, alternatively, some of the oligomers in the plurality may be the same and some may be different. When a plurality of oligomers are coupled to the drug, it may be preferable to couple one or more of the oligomers to the drug with hydrolyzable bonds and couple one or more of the oligomers to the drug with non-hydrolyzable bonds. Alternatively, all of the bonds coupling the plurality of oligomers to the drug may be hydrolyzable, but have varying degrees of hydrolyzability such that, for example, one or more of the oligomers is rapidly removed from the drug by hydrolysis in the body and one or more of the oligomers is slowly removed from the drug by hydrolysis in the body.

The oligomer may be coupled to the drug at various nucleophilic residues of the drug including, but not limited to, nucleophilic hydroxyl functions and/or amino functions. When the drug is a polypeptide, a nucleophilic hydroxyl function may be found, for example, at serine and/or tyrosine residues, and a nucleophilic amino function may be found, for example, at histidine and/or lysine residues, and/or at the one or more N-termini of the polypeptide. When an oligomer is coupled to the one or more N-termini of the polypeptide, the coupling preferably forms a secondary amine. For example, when the drug is human

insulin, the oligomer may be coupled to an amino functionality of the insulin including the amino functionality of Gly^{A1}, the amino functionality of Phe^{B1}, and the amino functionality of Lys^{B29}. When one oligomer is coupled to the human insulin, the oligomer is preferably coupled to the amino functionality of Lys^{B29}. When two oligomers are coupled to the human insulin, the oligomers are preferably coupled to the amino functionality of Phe^{B1} and the amino functionality of Lys^{B29}. While more than one oligomer may be coupled to the human insulin, a higher activity (improved glucose lowering ability) is observed for the mono-conjugated human insulin. As another example, when the drug is salmon calcitonin, the oligomer may be coupled to an amino functionality of the salmon calcitonin, including the amino functionality of Lys^{11}, Lys^{18} and the N-terminus. While one or more oligomers may be coupled to the salmon calcitonin, a higher activity (improved glucose lowering ability) is observed for the di-conjugated salmon calcitonin where an oligomer is coupled to the amino functionality of Lys^{11} and an oligomer is coupled to the amino functionality of Lys^{18}. As yet another example, when the drug is human growth hormone, the oligomer may be coupled to an amino functionality of Phe^{1}, Lys^{38}, Lys^{41}, Lys^{70}, Lys^{115}, Lys^{140}, Lys^{145}, Lys^{158}, Lys^{168}, and/or Lys^{172}.

Mixtures of drug oligomer conjugates where each conjugate in the mixture has the same number of polyethylene glycol subunits may be synthesized by various methods. For example, a mixture of oligomers consisting of carboxylic acid and polyethylene glycol where each oligomer in the mixture has the same number of polyethylene glycol subunits is synthesized by contacting a mixture of carboxylic acid with a mixture of polyethylene glycol where each polyethylene glycol molecule in the mixture has the same number of polyethylene glycol subunits under conditions sufficient to provide a mixture of oligomers where each oligomer in the mixture has the same number of polyethylene glycol subunits. The oligomers of the mixture where each oligomer in the mixture has the same number of polyethylene glycol subunits are then activated so that they are capable of reacting with a drug to provide a drug-oligomer conjugate. One embodiment of a synthesis route for providing a mixture of activated oligomers where each oligomer in the mixture has the same number of polyethylene glycol subunits is illustrated in **Figure 3** and described in Examples 11-18 hereinbelow. Another embodiment of a synthesis route for providing a mixture of activated oligomers where each oligomer in the mixture has the same number of polyethylene glycol subunits is illustrated in **Figure 4** and described in Examples 19-24 hereinbelow. Still

another embodiment of a synthesis route for providing a mixture of activated oligomers where each oligomer in the mixture has the same number of polyethylene glycol subunits is illustrated in **Figure 5** and described in Examples 25-29 hereinbelow. Yet another embodiment of a synthesis route for providing a mixture of activated oligomers where each oligomer in the mixture has the same number of polyethylene glycol subunits is illustrated in **Figure 6** and described in Examples 30-31 hereinbelow. Another embodiment of a synthesis route for providing a mixture of activated oligomers where each oligomer in the mixture has the same number of polyethylene glycol subunits is illustrated in **Figure 7** and described in Examples 32-37 hereinbelow. Still another embodiment of a synthesis route for providing a mixture of activated oligomers where each oligomer in the mixture has the same number of polyethylene glycol subunits is illustrated in **Figure 8** and described in Example 38 hereinbelow. Yet another embodiment of a synthesis route for providing a mixture of activated oligomers where each oligomer in the mixture has the same number of polyethylene glycol subunits is illustrated in **Figure 9** and described in Example 39 hereinbelow. Another embodiment of a synthesis route for providing a mixture of activated oligomers having a mixture of activated oligomers where each oligomer in the mixture has the same number of polyethylene glycol subunits is illustrated in **Figure 10** and described in Example 40 hereinbelow.

The mixture of activated oligomers where each oligomer in the mixture has the same number of polyethylene glycol subunits is reacted with a mixture of drugs under conditions sufficient to provide a mixture of drug-oligomer conjugates, as described, for example, in Examples 41-120 hereinbelow. As will be understood by those skilled in the art, the reaction conditions (e.g., selected molar ratios, solvent mixtures and/or pH) may be controlled such that the mixture of drug-oligomer conjugates resulting from the reaction of the mixture of activated oligomers where each oligomer in the mixture has the same number of polyethylene glycol subunits and the mixture of drugs is a mixture of conjugates where each conjugate in the mixture has the same number of polyethylene glycol subunits. For example, conjugation at the amino functionality of lysine may be suppressed by maintaining the pH of the reaction solution below the pK_a of lysine. Alternatively, the mixture of drug-oligomer conjugates may be separated and isolated utilizing, for example, HPLC to provide a mixture of drug-oligomer conjugates, for example mono-, di-, or tri-conjugates, where each conjugate in the mixture has the same number of polyethylene glycol subunits. The degree of conjugation (e.g.,

whether the isolated molecule is a mono-, di-, or tri-conjugate) of a particular isolated conjugate may be determined and/or verified utilizing various techniques as will be understood by those skilled in the art including, but not limited to, mass spectroscopy. The particular conjugate structure (e.g., whether the oligomer is at Gly^{A1}, Phe^{B1}, Lys^{B29} of a human insulin monoconjugate) may be determined and/or verified utilizing various techniques as will be understood by those skilled in the art including, but not limited to, sequence analysis, peptide mapping, selective enzymatic cleavage, and/or endopeptidase cleavage.

As will be understood by those skilled in the art, one or more of the reaction sites on the drug may be blocked by, for example, reacting the drug with a suitable blocking reagent such as N-tert-butoxycarbonyl (t-BOC), or N-(9-fluorenylmethoxycarbonyl) (N-FMOC). This process may be preferred, for example, when the drug is a polypeptide and it is desired to form an unsaturated conjugate (i.e., a conjugate wherein not all nucleophilic residues are conjugated) having an oligomer at the one or more N-termini of the polypeptide. Following such blocking, the mixture of blocked drugs may be reacted with the mixture of activated oligomers where each oligomer in the mixture has the same number of polyethylene glycol subunits to provide a mixture of drug-oligomer conjugates having oligomer(s) coupled to one or more nucleophilic residues and having blocking moieties coupled to other nucleophilic residues. After the conjugation reaction, the drug-oligomer conjugates may be de-blocked as will be understood by those skilled in the art. If necessary, the mixture of drug-oligomer conjugates may then be separated as described above to provide a mixture of drug-oligomer conjugates where each conjugate in the mixture has the same number of polyethylene glycol subunits. Alternatively, the mixture of drug-oligomer conjugates may be separated prior to de-blocking.

Mixtures of drug-oligomer conjugates where each conjugate in the mixture has the same number of polyethylene glycol subunits according to embodiments of the present invention preferably have improved properties when compared with those of conventional mixtures. For example, a mixture of drug-oligomer conjugates where each conjugate in the mixture has the same number of polyethylene glycol subunits preferably has an *in vivo* activity that is greater than the *in vivo* activity of a polydispersed mixture of drug-oligomer conjugates having the same number average molecular weight as the mixture of drug-oligomer conjugates where each conjugate in the mixture has the same number of

polyethylene glycol subunits. As will be understood by those skilled in the art, the number average molecular weight of the mixture of drug-oligomer conjugates where each conjugate in the mixture has the same number of polyethylene glycol subunits and the number average weight of the polydispersed mixture may be measured by various methods including, but not limited to, size exclusion chromatography such as gel permeation chromatography as described, for example, in H.R. Allcock & F.W. Lampe, CONTEMPORARY POLYMER CHEMISTRY 394-402 (2d. ed., 1991).

As another example, a mixture of drug-oligomer conjugates where each conjugate in the mixture has the same number of polyethylene glycol subunits preferably has an *in vitro* activity that is greater than the *in vitro* activity of a polydispersed mixture of drug-oligomer conjugates having the same number average molecular weight as the mixture of drug-oligomer conjugates where each conjugate in the mixture has the same number of polyethylene glycol subunits. As will be understood by those skilled in the art, the number average molecular weight of the mixture of drug-oligomer conjugates where each conjugate in the mixture has the same number of polyethylene glycol subunits and the number average weight of the polydispersed mixture may be measured by various methods including, but not limited to, size exclusion chromatography.

The *in vitro* activity of a particular mixture may be measured by various methods, as will be understood by those skilled in the art. Preferably, the *in vitro* activity is measured using a Cytosensor® Microphysiometer commercially available from Molecular Devices Corporation of Sunnyvale, California. The microphysiometer monitors small changes in the rates of extracellular acidification in response to a drug being added to cultured cells in a transwell. This response is proportional to the activity of the molecule under study.

As still another example, a mixture of drug-oligomer conjugates where each conjugate in the mixture has the same number of polyethylene glycol subunits preferably has an increased resistance to degradation by chymotrypsin when compared to the resistance to degradation by chymotrypsin of a polydispersed mixture of drug-oligomer conjugates having the same number average molecular weight as the mixture of drug-oligomer conjugates where each conjugate in the mixture has the same number of polyethylene glycol subunits. As will be understood by those skilled in the art, the number average molecular weight of the mixture of drug-oligomer conjugates where each conjugate in the mixture has the same number of polyethylene glycol subunits and the number average weight of the polydispersed

mixture may be measured by various methods including, but not limited to, size exclusion chromatography.

As yet another example, a mixture of drug-oligomer conjugates where each conjugate in the mixture has the same number of polyethylene glycol subunits preferably has an inter-subject variability that is less than the inter-subject variability of a polydispersed mixture of drug-oligomer conjugates having the same number average molecular weight as the mixture of drug-oligomer conjugates where each conjugate in the mixture has the same number of polyethylene glycol subunits. As will be understood by those skilled in the art, the number average molecular weight of the mixture of drug-oligomer conjugates where each conjugate in the mixture has the same number of polyethylene glycol subunits and the number average weight of the polydispersed mixture may be measured by various methods including, but not limited to, size exclusion chromatography. The inter-subject variability may be measured by various methods as will be understood by those skilled in the art. The inter-subject variability is preferably calculated as follows. The area under a dose response curve (AUC) (i.e., the area between the dose-response curve and a baseline value) is determined for each subject. The average AUC for all subjects is determined by summing the AUCs of each subject and dividing the sum by the number of subjects. The absolute value of the difference between the subject's AUC and the average AUC is then determined for each subject. The absolute values of the differences obtained are then summed to give a value that represents the inter-subject variability. Lower values represent lower inter-subject variabilities and higher values represent higher inter-subject variabilities.

Mixtures of drug-oligomer conjugates where each conjugate in the mixture has the same number of polyethylene glycol subunits according to embodiments of the present invention preferably have two or more of the above-described improved properties. More preferably, mixtures of drug-oligomer conjugates where each conjugate in the mixture has the same number of polyethylene glycol subunits according to embodiments of the present invention have three or more of the above-described improved properties. Most preferably, mixtures of drug-oligomer conjugates where each conjugate in the mixture has the same number of polyethylene glycol subunits according to embodiments of the present invention have all four of the above-described improved properties.

According to still other embodiments of the present invention, a mixture of conjugates is provided in which each conjugate has the same molecular weight and has the formula:

$$\mathrm{Drug}\left[B-L_h-G_i-R_m-G'_j-R'_n-G''_k-T\right]_p \qquad (A)$$

wherein:

B is a bonding moiety;

L is a linker moiety;

G, G' and G" are individually selected spacer moieties;

R is a lipophilic moiety and R' is a polyalkylene glycol moiety, or R' is the lipophilic moiety and R is the polyalkylene glycol moiety;

T is a terminating moiety;

h, i, j, k, m and n are individually 0 or 1, with the proviso that when R is the polyalkylene glycol moiety; m is 1, and when R' is the polyalkylene glycol moiety, n is 1; and

p is an integer from 1 to the number of nucleophilic residues on the drug.

According to these embodiments of the present invention, R or R' is a polyalkylene moiety. The oligomer may be various oligomers comprising a polyalkylene glycol moiety as will be understood by those skilled in the art. Preferably, the polyalkylene glycol moiety has at least 2, 3, or 4 polyalkylene glycol subunits. More preferably, the polyalkylene glycol moiety has at least 5 or 6 polyalkylene glycol subunits. Most preferably, the polyalkylene glycol moiety of the oligomer has at least 7 polyalkylene glycol subunits. The polyalkylene glycol moiety of the oligomer is preferably a lower alkyl polyalkylene glycol moiety such as a polyethylene glycol moiety, a polypropylene glycol moiety, or a polybutylene glycol moiety. When the polyalkylene moiety is a polypropylene glycol moiety, the polypropylene glycol moiety preferably has a uniform structure. An exemplary polypropylene glycol moiety having a uniform structure is as follows:

$$-O-\underset{\displaystyle CH_3}{\underset{|}{CH}}-CH_2-O-\underset{\displaystyle CH_3}{\underset{|}{CH}}-CH_2-O-\underset{\displaystyle CH_3}{\underset{|}{CH}}-CH_2-O-\underset{\displaystyle CH_3}{\underset{|}{CH}}-CH_2-O-$$

This uniform polypropylene glycol structure may be described as having only one methyl substituted carbon atom adjacent each oxygen atom in the polypropylene glycol chain. Such uniform polypropylene glycol moieties may exhibit both lipophilic and hydrophilic characteristics and thus be useful in providing amphiphilic drug-oligomer conjugates without the use of lipophilic polymer moieties (i.e., the sum of m + n is 1). Furthermore, coupling the secondary alcohol moiety of the polypropylene glycol moiety with a drug may provide the

drug (e.g., a polypeptide) with improved resistance to degradation caused by enzymes such as trypsin and chymotrypsin found, for example, in the gut.

Uniform polypropylene glycol according to embodiments of the present invention is preferably synthesized as illustrated in **Figures 11** through 13, which will now be described. As illustrated in **Figure** 11, 1,2-propanediol **53** is reacted with a primary alcohol blocking reagent to provide a secondary alcohol extension monomer **54**. The primary alcohol blocking reagent may be various primary alcohol blocking reagents as will be understood by those skilled in the art including, but not limited to, silylchloride compounds such as t-butyldiphenylsilylchloride and t-butyldimethylsilylchloride, and esterification reagents such as Ac_2O. Preferably, the primary alcohol blocking reagent is a primary alcohol blocking reagent that is substantially non-reactive with secondary alcohols, such as t-butyldiphenylsilylchloride or t-butyldimethylsilylchloride. The secondary alcohol extension monomer (**54**) may be reacted with methanesulfonyl chloride ($MeSO_2Cl$) to provide a primary extension alcohol monomer mesylate **55**.

Alternatively, the secondary alcohol extension monomer **54** may be reacted with a secondary alcohol blocking reagent to provide compound **56**. The secondary alcohol blocking reagent may be various secondary alcohol blocking reagents as will be understood by those skilled in the art including, but not limited to, benzyl chloride. The compound **56** may be reacted with a B_1 de-blocking reagent to remove the blocking moiety B_1 and provide a primary alcohol extension monomer **57**. The B_1 de-blocking reagent may be selected from various de-blocking reagents as will be understood by one skilled in the art. When the primary alcohol has been blocked by forming an ester, the B_1 de-blocking reagent is a de-esterification reagent, such as a base (e.g., potassium carbonate). When the primary alcohol has been blocked using a silylchloride, the B_1 de-blocking reagent is preferably tetrabutylammonium fluoride (TBAF). The primary alcohol extension monomer **57** may be reacted with methane sulfonyl chloride to provide a secondary alcohol extension monomer mesylate **58**.

The primary alcohol extension monomer **54** and the secondary alcohol extension monomer **57** may be capped as follows. The secondary alcohol extension monomer **54** may be reacted with a capping reagent to provide a compound **59**. The capping reagent may be various capping reagents as will be understood by those skilled in the art including, but not limited to, alkyl halides such as methyl chloride. The compound **59** may be reacted with a B_1

de-blocking agent as described above to provide a primary alcohol capping monomer 60. The primary alcohol capping monomer 60 may be reacted with methane sulfonyl chloride to provide the secondary alcohol capping monomer mesylate 61. The primary alcohol extension monomer 57 may be reacted with a capping reagent to provide a compound 62. The capping reagent may be various capping reagents as described above. The compound 62 may be reacted with a B_2 de-blocking reagent to remove the blocking moiety B_2 and provide a secondary alcohol capping monomer 63. The B_2 de-blocking reagent may be various de-blocking agents as will be understood by those skilled in the art including, but not limited to, H_2 in the presence of a palladium/activated carbon catalyst. The secondary alcohol capping monomer may be reacted with methanesulfonyl chloride to provide a primary alcohol capping monomer mesylate 64. While the embodiments illustrated in **Figure** 11 show the synthesis of capping monomers, it is to be understood that similar reactions may be performed to provide capping polymers.

In general, chain extensions may be effected by reacting a primary alcohol extension mono- or poly-mer such as the primary alcohol extension monomer 57 with a primary alcohol extension mono- or poly-mer mesylate such as the primary alcohol extension monomer mesylate 55 to provide various uniform polypropylene chains or by reacting a secondary alcohol extension mono- or poly-mer such as the secondary alcohol extension monomer 54 with a secondary alcohol extension mono-or poly-mer mesylate such as the secondary alcohol extension monomer mesylate 58.

For example, in **Figure** 13, the primary alcohol extension monomer mesylate 55 is reacted with the primary alcohol extension monomer 57 to provide a dimer compound 65. Alternatively, the secondary alcohol extension monomer mesylate 58 may be reacted with the secondary alcohol extension monomer 54 to provide the dimer compound 65. The B_1 blocking moiety on the dimer compound 65 may be removed using a B_1 de-blocking reagent as described above to provide a primary alcohol extension dimer 66. The primary alcohol extension dimer 66 may be reacted with methane sulfonyl chloride to provide a secondary alcohol extension dimer mesylate 67. Alternatively, the B_2 blocking moiety on the dimer compound 65 may be removed using the B_2 de-blocking reagent as described above to provide a secondary alcohol extension dimer 69. The secondary alcohol extension dimer 69 may be reacted with methane sulfonyl chloride to provide a primary alcohol extension dimer mesylate 70.

As will be understood by those skilled in the art, the chain extension process may be repeated to achieve various other chain lengths. For example, as illustrated in **Figure 13**, the primary alcohol extension dimer 66 may be reacted with the primary alcohol extension dimer mesylate 70 to provide a tetramer compound 72. As further illustrated in **Figure 13**, a generic chain extension reaction scheme involves reacting the primary alcohol extension mono- or poly-mer 73 with the primary alcohol extension mono- or poly-mer mesylate 74 to provide the uniform polypropylene polymer 75. The values of m and n may each range from 0 to 1000 or more. Preferably, m and n are each from 0 to 50. While the embodiments illustrated in **Figure 13** show primary alcohol extension mono- and/or poly-mers being reacted with primary alcohol extension mono- and/or poly-mer mesylates, it is to be understood that similar reactions may be carried out using secondary alcohol extension mono- and/or poly-mers and secondary alcohol extension mono- and/or poly-mer mesylates.

An end of a primary alcohol extension mono- or poly-mer or an end of a primary alcohol extension mono- or poly-mer mesylate may be reacted with a primary alcohol capping mono- or poly-mer mesylate or a primary alcohol capping mono- or poly-mer, respectively, to provide a capped uniform polypropylene chain. For example, as illustrated in **Figure 12**, the primary alcohol extension dimer mesylate 70 is reacted with the primary alcohol capping monomer 60 to provide the capped/blocked primary alcohol extension trimer 71. As will be understood by those skilled in the art, the B_1 blocking moiety may be removed and the resulting capped primary alcohol extension trimer may be reacted with a primary alcohol extension mono- or poly-mer mesylate to extend the chain of the capped trimer 71.

An end of a secondary alcohol extension mono-or poly-mer or an end of a secondary alcohol extension mono-or poly-mer mesylate may be reacted with a secondary alcohol capping mono-or poly-mer mesylate or a secondary alcohol capping mono- or poly-mer, respectively, to provide a capped uniform polypropylene chain. For example, as illustrated in **Figure 12**, the secondary alcohol extension dimer mesylate 67 is reacted with the secondary alcohol capping monomer 63 to provide the capped/blocked primary alcohol extension trimer 68. The B_2 blocking moiety may be removed as described above and the resulting capped secondary alcohol extension trimer may be reacted with a secondary alcohol extension mer mesylate to extend the chain of the capped trimer 68. While the syntheses illustrated in **Figure 12** show the reaction of a dimer with a capping monomer to provide a trimer, it is to be understood that the capping process may be performed at any point in the synthesis of a

uniform polypropylene glycol moiety, or, alternatively, uniform polypropylene glycol moieties may be provided that are not capped. While the embodiments illustrated in **Figure 12** show the capping of a polybutylene oligomer by synthesis with a capping monomer, it is to be understood that polybutylene oligomers of the present invention may be capped directly (i.e., without the addition of a capping monomer) using a capping reagent as described above in **Figure 11**.

Uniform polypropylene glycol moieties according to embodiments of the present invention may be coupled to a drug, a lipophilic moiety such as a carboxylic acid, and/or various other moieties by various methods as will be understood by those skilled in the art including, but not limited to, those described herein with respect to polyethylene glycol moieties.

According to these embodiments of the present invention, R or R' is a lipophilic moiety as will be understood by those skilled in the art. The lipophilic moiety is preferably a saturated or unsaturated, linear or branched alkyl moiety or a saturated or unsaturated, linear or branched fatty acid moiety. When the lipophilic moiety is an alkyl moiety, it is preferably a linear, saturated or unsaturated alkyl moiety having 1 to 28 carbon atoms. More preferably, the alkyl moiety has 2 to 12 carbon atoms. When the lipophilic moiety is a fatty acid moiety, it is preferably a natural fatty acid moiety that is linear, saturated or unsaturated, having 2 to 18 carbon atoms. More preferably, the fatty acid moiety has 3 to 14 carbon atoms. Most preferably, the fatty acid moiety has at least 4, 5 or 6 carbon atoms.

According to these embodiments of the present invention, the spacer moieties, G, G' and G", are spacer moieties as will be understood by those skilled in the art. Spacer moieties are preferably selected from the group consisting of sugar, cholesterol and glycerine moieties. Preferably, oligomers of these embodiments do not include spacer moieties (i.e., i, j and k are preferably 0).

According to these embodiments of the present invention, the linker moiety, L, may be used to couple the oligomer with the drug as will be understood by those skilled in the art. Linker moieties are preferably selected from the group consisting of alkyl and fatty acid.

According to these embodiments of the present invention, the terminating moiety is preferably an alkyl or alkoxy moiety, and is more preferably a lower alkyl or lower alkoxy moiety. Most preferably, the terminating moiety is methyl or methoxy. While the terminating moiety is preferably an alkyl or alkoxy moiety, it is to be understood that the

terminating moiety may be various moieties as will be understood by those skilled in the art including, but not limited to, sugars, cholesterol, alcohols, and fatty acids.

According to these embodiments of the present invention, the oligomer, which is represented by the bracketed portion of the structure of Formula A, is covalently coupled to the drug. In some embodiments, the drug is coupled to the oligomer utilizing a hydrolyzable bond (e.g., an ester or carbonate bond). A hydrolyzable coupling may provide a drug-oligomer conjugate that acts as a prodrug. In certain instances, for example where the drug-oligomer conjugate is inactive (i.e., the conjugate lacks the ability to affect the body through the drug's primary mechanism of action), a hydrolyzable coupling may provide for a time-release or controlled-release effect, administering the drug over a given time period as one or more oligomers are cleaved from their respective drug-oligomer conjugates to provide the active drug. In other embodiments, the drug is coupled to the oligomer utilizing a non-hydrolyzable bond (e.g., a carbamate, amide, or ether bond). Use of a non-hydrolyzable bond may be preferable when it is desirable to allow the drug-oligomer conjugate to circulate in the bloodstream for an extended period of time, preferably at least 2 hours. The bonding moiety, B, may be various bonding moieties that may be used to covalently couple the oligomer with the drug as will be understood by those skilled in the art. Bonding moieties are preferably selected from the group consisting of covalent bond(s), ester moieties, carbonate moieties, carbamate moieties, amide moieties and secondary amine moieties.

The variable p is an integer from 1 to the number of nucleophilic residues on the drug. When p is greater than 1, more than one oligomer (i.e., a plurality of oligomers) is coupled to the drug. According to these embodiments of the present invention, the oligomers in the plurality are the same.

The oligomer may be coupled to the drug at various nucleophilic residues of the drug including, but not limited to, nucleophilic hydroxyl functions and/or amino functions. When the drug is a polypeptide, a nucleophilic hydroxyl function may be found, for example, at serine and/or tyrosine residues, and a nucleophilic amino function may be found, for example, at histidine and/or lysine residues, and/or at the one or more N-termini of the polypeptide. When an oligomer is coupled to the one or more N-termini of the polypeptide, the coupling preferably forms a secondary amine. For example, when the drug is human insulin, the oligomer may be coupled to an amino functionality of the insulin including the amino functionality of Gly^{A1}, the amino functionality of Phe^{B1}, and the amino functionality of

Lys^{B29}. When one oligomer is coupled to the human insulin, the oligomer is preferably coupled to the amino functionality of Lys^{B29}. When two oligomers are coupled to the human insulin, the oligomers are preferably coupled to the amino functionality of Phe^{B1} and the amino functionality of Lys^{B29}. While more than one oligomer may be coupled to the human insulin, a higher activity (improved glucose lowering ability) is observed for the mono-conjugated human insulin. As another example, when the drug is salmon calcitonin, the oligomer may be coupled to an amino functionality of the salmon calcitonin, including the amino functionality of Lys^{11}, Lys^{18} and the N-terminus. While one or more oligomers may be coupled to the salmon calcitonin, a higher activity (improved glucose lowering ability) is observed for the di-conjugated salmon calcitonin where an oligomer is coupled to the amino functionality of Lys^{11} and an oligomer is coupled to the amino functionality of Lys^{18}. As yet another example, when the drug is human growth hormone, the oligomer may be coupled to an amino functionality of Phe^{1}, Lys^{38}, Lys^{41}, Lys^{70}, Lys^{115}, Lys^{140}, Lys^{145}, Lys^{158}, Lys^{168}, and/or Lys^{172}.

Mixtures of drug-oligomer conjugates where each conjugate in the mixture has the same molecular weight and has the structure of Formula A may be synthesized by various methods. For example, a mixture of oligomers consisting of carboxylic acid and polyethylene glycol is synthesized by contacting a mixture of carboxylic acid with a mixture of polyethylene glycol under conditions sufficient to provide a mixture of oligomers. The oligomers of the mixture are then activated so that they are capable of reacting with a drug to provide a drug-oligomer conjugate. One embodiment of a synthesis route for providing a mixture of activated oligomers where each oligomer has the same molecular weight and has a structure of the oligomer of Formula A is illustrated in **Figure 3** and described in Examples 11-18 hereinbelow. Another embodiment of a synthesis route for providing a mixture of activated oligomers where each oligomer has the same molecular weight and has a structure of the oligomer of Formula A is illustrated in **Figure 4** and described in Examples 19-24 hereinbelow. Still another embodiment of a synthesis route for providing a mixture of activated oligomers where each oligomer has the same molecular weight and has a structure of the oligomer of Formula A is illustrated in **Figure 5** and described in Examples 25-29 hereinbelow. Yet another embodiment of a synthesis route for providing a mixture of activated oligomers where each oligomer has the same molecular weight and has a structure of the oligomer of Formula A is illustrated in **Figure 6** and described in Examples 30-31

hereinbelow. Another embodiment of a synthesis route for providing a mixture of activated oligomers where each oligomer has the same molecular weight and has a structure of the oligomer of Formula A is illustrated in **Figure 7** and described in Examples 32-37 hereinbelow. Still another embodiment of a synthesis route for providing a mixture of activated oligomers where each oligomer has the same molecular weight and has a structure of the oligomer of Formula A is illustrated in **Figure 8** and described in Example 38 hereinbelow. Yet another embodiment of a synthesis route for providing a mixture of activated oligomers where each oligomer has the same molecular weight and has a structure of the oligomer of Formula A is illustrated in **Figure 9** and described in Example 39 hereinbelow. Another embodiment of a synthesis route for providing a mixture of activated oligomers where each oligomer has the same molecular weight and has a structure of the oligomer of Formula A is illustrated in **Figure 10** and described in Example 40 hereinbelow.

The mixture of activated oligomers where each oligomer has the same molecular weight and has a structure of the oligomer of Formula A is reacted with a mixture of drugs where each drug in the mixture has the same molecular weight under conditions sufficient to provide a mixture of drug-oligomer conjugates, as described, for example, in Examples 41-120 hereinbelow. As will be understood by those skilled in the art, the reaction conditions (e.g., selected molar ratios, solvent mixtures and/or pH) may be controlled such that the mixture of drug-oligomer conjugates resulting from the reaction of the mixture of activated oligomers where each oligomer has the same molecular weight and has a structure of the oligomer of Formula A and the mixture of drugs is a mixture of conjugates where each conjugate has the same molecular weight and has the structure Formula A. For example, conjugation at the amino functionality of lysine may be suppressed by maintaining the pH of the reaction solution below the pK_a of lysine. Alternatively, the mixture of drug-oligomer conjugates may be separated and isolated utilizing, for example, HPLC to provide a mixture of drug-oligomer conjugates, for example mono-, di-, or tri-conjugates, where each conjugate in the mixture has the same number molecular weight and has the structure of Formula A. The degree of conjugation (e.g., whether the isolated molecule is a mono-, di-, or tri-conjugate) of a particular isolated conjugate may be determined and/or verified utilizing various techniques as will be understood by those skilled in the art including, but not limited to, mass spectroscopy. The particular conjugate structure (e.g., whether the oligomer is at Gly^{A1}, Phe^{B1}, or Lys^{B29} of a human insulin monoconjugate) may be determined and/or

verified utilizing various techniques as will be understood by those skilled in the art including, but not limited to, sequence analysis, peptide mapping, selective enzymatic cleavage, and/or endopeptidase cleavage.

As will be understood by those skilled in the art, one or more of the reaction sites on the drug may be blocked by, for example, reacting the drug with a suitable blocking reagent such as N-tert-butoxycarbonyl (t-BOC), or N-(9-fluorenylmethoxycarbonyl) (N-FMOC). This process may be preferred, for example, when the drug is a polypeptide and it is desired to form an unsaturated conjugate (i.e., a conjugate wherein not all nucleophilic residues are conjugated) having an oligomer at the one or more N-termini of the polypeptide. Following such blocking, the mixture of blocked drugs may be reacted with the mixture of activated oligomers where each oligomer in the mixture has the same molecular weight and has a structure of the oligomer of Formula A to provide a mixture of drug-oligomer conjugates having oligomer(s) coupled to one or more nucleophilic residues and having blocking moieties coupled to other nucleophilic residues. After the conjugation reaction, the drug-oligomer conjugates may be de-blocked as will be understood by those skilled in the art. If necessary, the mixture of drug-oligomer conjugates may then be separated as described above to provide a mixture of drug-oligomer conjugates where each conjugate in the mixture has the same molecular weight and has the structure of Formula A. Alternatively, the mixture of drug-oligomer conjugates may be separated prior to de-blocking.

Mixtures of drug-oligomer conjugates where each conjugate in the mixture has the same molecular weight and has the structure of Formula A according to embodiments of the present invention preferably have improved properties when compared with those of conventional mixtures. For example, a mixture of drug-oligomer conjugates where each conjugate in the mixture has the same molecular weight and has the structure of Formula A preferably has an *in vivo* activity that is greater than the *in vivo* activity of a polydispersed mixture of drug-oligomer conjugates having the same number average molecular weight as the mixture of drug-oligomer conjugates where each conjugate in the mixture has the same molecular weight and has the structure of Formula A. As will be understood by those skilled in the art, the number average molecular weight of the mixture of drug-oligomer conjugates where each conjugate in the mixture has the same molecular weight and has the structure of Formula A and the number average weight of the polydispersed mixture may be measured by various methods including, but not limited to, size exclusion chromatography such as gel

permeation chromatography as described, for example, in H.R. Allcock & F.W. Lampe, CONTEMPORARY POLYMER CHEMISTRY 394-402 (2d. ed., 1991).

As another example, a mixture of drug-oligomer conjugates where each conjugate in the mixture has the same molecular weight and has the structure of Formula A preferably has an *in vitro* activity that is greater than the *in vitro* activity of a polydispersed mixture of drug-oligomer conjugates having the same number average molecular weight as the mixture of drug-oligomer conjugates where each conjugate in the mixture has the same molecular weight and has the structure of Formula A. As will be understood by those skilled in the art, the number average molecular weight of the mixture of drug-oligomer conjugates where each conjugate in the mixture has the same molecular weight and has the structure of Formula A and the number average weight of the polydispersed mixture may be measured by various methods including, but not limited to, size exclusion chromatography.

The *in vitro* activity of a particular mixture may be measured by various methods, as will be understood by those skilled in the art. Preferably, the *in vitro* activity is measured using a Cytosensor® Microphysiometer commercially available from Molecular Devices Corporation of Sunnyvale, California. The microphysiometer monitors small changes in the rates of extracellular acidification in response to a drug being added to cultured cells in a transwell. This response is proportional to the activity of the molecule under study.

As still another example, a mixture of drug-oligomer conjugates where each conjugate in the mixture has the same molecular weight and has the structure of Formula A preferably has an increased resistance to degradation by chymotrypsin when compared to the resistance to degradation by chymotrypsin of a polydispersed mixture of drug-oligomer conjugates having the same number average molecular weight as the mixture of drug-oligomer conjugates where each conjugate in the mixture has the same molecular weight and has the structure of Formula A. As will be understood by those skilled in the art, the number average molecular weight of the mixture of drug-oligomer conjugates where each conjugate in the mixture has the same molecular weight and has the structure of Formula A and the number average weight of the polydispersed mixture may be measured by various methods including, but not limited to, size exclusion chromatography.

As yet another example, a mixture of drug-oligomer conjugates where each conjugate in the mixture has the same molecular weight and has the structure of Formula A preferably has an inter-subject variability that is less than the inter-subject variability of a polydispersed

mixture of drug-oligomer conjugates having the same number average molecular weight as the mixture of drug-oligomer conjugates where each conjugate in the mixture has the same molecular weight and has the structure of Formula A. As will be understood by those skilled in the art, the number average molecular weight of the mixture of drug-oligomer conjugates where each conjugate in the mixture has the same molecular weight and has the structure of Formula A and the number average weight of the polydispersed mixture may be measured by various methods including, but not limited to, size exclusion chromatography. The inter-subject variability may be measured by various methods as will be understood by those skilled in the art. The inter-subject variability is preferably calculated as follows. The area under a dose response curve (AUC) (i.e., the area between the dose-response curve and a baseline value) is determined for each subject. The average AUC for all subjects is determined by summing the AUCs of each subject and dividing the sum by the number of subjects. The absolute value of the difference between the subject's AUC and the average AUC is then determined for each subject. The absolute values of the differences obtained are then summed to give a value that represents the inter-subject variability. Lower values represent lower inter-subject variabilities and higher values represent higher inter-subject variabilities.

Mixtures of drug-oligomer conjugates where each conjugate in the mixture has the same molecular weight and has the structure of Formula A according to embodiments of the present invention preferably have two or more of the above-described improved properties. More preferably, mixtures of drug-oligomer conjugates where each conjugate in the mixture has the same molecular weight and has the structure of Formula A according to embodiments of the present invention have three or more of the above-described improved properties. Most preferably, mixtures of drug-oligomer conjugates where each conjugate in the mixture has the same molecular weight and has the structure of Formula A according to embodiments of the present invention have all four of the above-described improved properties.

Pharmaceutical compositions comprising a conjugate mixture according to embodiments of the present invention are also provided. The mixtures of drug-oligomer conjugates described above may be formulated for administration in a pharmaceutical carrier in accordance with known techniques. *See, e.g.*, Remington, *The Science And Practice of Pharmacy* (9^{th} Ed. 1995). In the manufacture of a pharmaceutical composition according to embodiments of the present invention, the mixture of drug-oligomer conjugates is typically

admixed with, *inter alia*, a pharmaceutically acceptable carrier. The carrier must, of course, be acceptable in the sense of being compatible with any other ingredients in the pharmaceutical composition and should not be deleterious to the patient. The carrier may be a solid or a liquid, or both, and is preferably formulated with the mixture of drug-oligomer conjugates as a unit-dose formulation, for example, a tablet, which may contain from about 0.01 or 0.5% to about 95% or 99% by weight of the mixture of drug-oligomer conjugates. The pharmaceutical compositions may be prepared by any of the well known techniques of pharmacy including, but not limited to, admixing the components, optionally including one or more accessory ingredients.

The pharmaceutical compositions according to embodiments of the present invention include those suitable for oral, rectal, topical, inhalation (e.g., via an aerosol) buccal (e.g., sub-lingual), vaginal, parenteral (e.g., subcutaneous, intramuscular, intradermal, intraarticular, intrapleural, intraperitoneal, inracerebral, intraarterial, or intravenous), topical (i.e., both skin and mucosal surfaces, including airway surfaces) and transdermal administration, although the most suitable route in any given case will depend on the nature and severity of the condition being treated and on the nature of the particular mixture of drug-oligomer conjugates which is being used.

Pharmaceutical compositions suitable for oral administration may be presented in discrete units, such as capsules, cachets, lozenges, or tables, each containing a predetermined amount of the mixture of drug-oligomer conjugates; as a powder or granules; as a solution or a suspension in an aqueous or non-aqueous liquid; or as an oil-in-water or water-in-oil emulsion. Such formulations may be prepared by any suitable method of pharmacy which includes the step of bringing into association the mixture of drug-oligomer conjugates and a suitable carrier (which may contain one or more accessory ingredients as noted above). In general, the pharmaceutical composition according to embodiments of the present invention are prepared by uniformly and intimately admixing the mixture of drug-oligomer conjugates with a liquid or finely divided solid carrier, or both, and then, if necessary, shaping the resulting mixture. For example, a tablet may be prepared by compressing or molding a powder or granules containing the mixture of drug-oligomer conjugates, optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing, in a suitable machine, the mixture in a free-flowing form, such as a powder or granules optionally mixed with a binder, lubricant, inert diluent, and/or surface active/dispersing agent(s).

Molded tablets may be made by molding, in a suitable machine, the powdered compound moistened with an inert liquid binder.

Pharmaceutical compositions suitable for buccal (sub-lingual) administration include lozenges comprising the mixture of drug-oligomer conjugates in a flavoured base, usually sucrose and acacia or tragacanth; and pastilles comprising the mixture of drug-oligomer conjugates in an inert base such as gelatin and glycerin or sucrose and acacia.

Pharmaceutical compositions according to embodiments of the present invention suitable for parenteral administration comprise sterile aqueous and non-aqueous injection solutions of the mixture of drug-oligomer conjugates, which preparations are preferably isotonic with the blood of the intended recipient. These preparations may contain anti-oxidants, buffers, bacteriostats and solutes which render the composition isotonic with the blood of the intended recipient. Aqueous and non-aqueous sterile suspensions may include suspending agents and thickening agents. The compositions may be presented in unit\dose or multi-dose containers, for example sealed ampoules and vials, and may be stored in a freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid carrier, for example, saline or water-for-injection immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules and tablets of the kind previously described. For example, an injectable, stable, sterile composition comprising a mixture of drug-oligomer conjugates in a unit dosage form in a sealed container may be provided. The mixture of drug-oligomer conjugates is provided in the form of a lyophilizate which is capable of being reconstituted with a suitable pharmaceutically acceptable carrier to form a liquid composition suitable for injection thereof into a subject. The unit dosage form typically comprises from about 10 mg to about 10 grams of the mixture of drug-oligomer conjugates. When the mixture of drug-oligomer conjugates is substantially water-insoluble, a sufficient amount of emulsifying agent which is physiologically acceptable may be employed in sufficient quantity to emulsify the mixture of drug-oligomer conjugates in an aqueous carrier. One such useful emulsifying agent is phosphatidyl choline.

Pharmaceutical compositions suitable for rectal administration are preferably presented as unit dose suppositories. These may be prepared by admixing the mixture of drug-oligomer conjugates with one or more conventional solid carriers, for example, cocoa butter, and then shaping the resulting mixture.

Pharmaceutical compositions suitable for topical application to the skin preferably take the form of an ointment, cream, lotion, paste, gel, spray, aerosol, or oil. Carriers which may be used include petroleum jelly, lanoline, polyethylene glycols, alcohols, transdermal enhancers, and combinations of two or more thereof.

Pharmaceutical compositions suitable for transdermal administration may be presented as discrete patches adapted to remain in intimate contact with the epidermis of the recipient for a prolonged period of time. Compositions suitable for transdermal administration may also be delivered by iontophoresis *(see, for example, Pharmaceutical Research* 3 (6):318 (1986)) and typically take the form of an optionally buffered aqueous solution of the mixture of drug-oligomer conjugates. Suitable formulations comprise citrate or bis\tris buffer (pH 6) or ethanol/water and contain from 0.1 to 0.2M active ingredient.

Methods of treating an insulin deficiency in a subject in need of such treatment by administering an effective amount of such pharmaceutical compositions are also provided. The effective amount of any mixture of drug-oligomer conjugates, the use of which is in the scope of present invention, will vary somewhat from mixture to mixture, and patient to patient, and will depend upon factors such as the age and condition of the patient and the route of delivery. Such dosages can be determined in accordance with routine pharmacological procedures known to those skilled in the art. As a general proposition, a dosage from about 0.1 to about 50 mg/kg will have therapeutic efficacy, with all weights being calculated based upon the weight of the mixture of drug-oligomer conjugates. Toxicity concerns at the higher level may restrict intravenous dosages to a lower level such as up to about 10 mg/kg, with all weights being calculated based upon the weight of the active base. A dosage from about 10 mg/kg to about 50 mg/kg may be employed for oral administration. Typically, a dosage from about 0.5 mg/kg to 5 mg/kg may be employed for intramuscular injection. The frequency of administration is usually one, two, or three times per day or as necessary to control the condition. Alternatively, the drug-oligomer conjugates may be administered by continuous infusion. The duration of treatment depends on the type of insulin deficiency being treated and may be for as long as the life of the patient.

Methods of synthesizing conjugate mixtures according to embodiments of the present invention are also provided. While the following embodiments of a synthesis route are directed to synthesis of a substantially monodispersed mixture, similar synthesis routes may

be utilized for synthesizing other drug-oligomer conjugate mixtures according to embodiments of the present invention.

A substantially monodispersed mixture of polymers comprising polyethylene glycol moieties is provided as illustrated in reaction 1:

$$\underset{(I)}{R^1(OC_2H_4)_nO^-X^+} + \underset{(II)}{R^2(OC_2H_4)_mOMs} \longrightarrow \underset{(III)}{R^2(OC_2H_4)_{m+n}OR^1} \qquad 1$$

R^1 is H or a lipophilic moiety. R^1 is preferably H, alkyl, aryl alkyl, an aromatic moiety, a fatty acid moiety, an ester of a fatty acid moiety, cholesteryl, or adamantyl. R^1 is more preferably H, lower alkyl, or an aromatic moiety. R^1 is most preferably H, methyl, or benzyl.

In Formula I, n is from 1 to 25. Preferably n is from 1 to 6.

X^+ is a positive ion. Preferably X^+ is any positive ion in a compound, such as a strong base, that is capable of ionizing a hydroxyl moiety on PEG. Examples of positive ions include, but are not limited to, sodium ions, potassium ions, lithium ions, cesium ions, and thallium ions.

R^2 is H or a lipophilic moiety. R^2 is preferably linear or branched alkyl, aryl alkyl, an aromatic moiety, a fatty acid moiety, or an ester of a fatty acid moiety. R^2 is more preferably lower alkyl, benzyl, a fatty acid moiety having 1 to 24 carbon atoms, or an ester of a fatty acid moiety having 1 to 24 carbon atoms. R^2 is most preferably methyl, a fatty acid moiety having 1 to 18 carbon atoms or an ethyl ester of a fatty acid moiety having 1 to 18 carbon atoms.

In Formula II, m is from 1 to 25. Preferably m is from 1 to 6.

Ms is a mesylate moiety (i.e., $CH_3S(O_2)$-).

As illustrated in reaction 1, a mixture of compounds having the structure of Formula I is reacted with a mixture of compounds having the structure of Formula II to provide a mixture of polymers comprising polyethylene glycol moieties and having the structure of Formula III. The mixture of compounds having the structure of Formula I is a substantially monodispersed mixture. Preferably, at least about 96, 97, 98 or 99 percent of the compounds in the mixture of compounds of Formula I have the same molecular weight, and, more preferably, the mixture of compounds of Formula I is a monodispersed mixture. The mixture of compounds of Formula II is a substantially monodispersed mixture. Preferably, at least about 96, 97, 98 or 99 percent of the compounds in the mixture of compounds of Formula II

have the same molecular weight, and, more preferably, the mixture of compounds of Formula II is a monodispersed mixture. The mixture of compounds of Formula III is a substantially monodispersed mixture. Preferably, at least about 96, 97, 98 or 99 percent of the compounds in the mixture of compound of Formula III have the same molecular weight. More preferably, the mixture of compounds of Formula III is a monodispersed mixture.

Reaction 1 is preferably performed between about 0°C and about 40°C, is more preferably performed between about 15°C and about 35°C, and is most preferably performed at room temperature (approximately 25°C).

Reaction 1 may be performed for various periods of time as will be understood by those skilled in the art. Reaction 1 is preferably performed for a period of time between about 0.25, 0.5 or 0.75 hours and about 2, 4 or 8 hours.

Reaction 1 is preferably carried out in an aprotic solvent such as, but not limited to, N,N-dimethylacetamide (DMA), N,N-dimethylformamide (DMF), dimethyl sulfoxide (DMSO), hexamethylphosphoric triamide, tetrahydrofuran (THF), dioxane, diethyl ether, methyl t-butyl ether (MTBE), toluene, benzene, hexane, pentane, N-methylpyrollidinone, tetrahydronaphthalene, decahydronaphthalene, 1,2-dichlorobenzene, 1,3-dimethyl-2-imidazolidinone, or a mixture thereof. More preferably, the solvent is DMF, DMA or toluene.

The molar ratio of the compound of Formula I to the compound of Formula II is preferably greater than about 1:1. More preferably, the molar ratio is at least about 2:1. By providing an excess of the compounds of Formula I, one can ensure that substantially all of the compounds of Formula II are reacted, which may aid in the recovery of the compounds of Formula III as discussed below.

Compounds of Formula I are preferably prepared as illustrated in reaction 2:

$$\underset{\text{(IV)}}{R^1(OC_2H_4)_nOH} \quad + \quad \xrightarrow[\text{on the PEG moiety of Formula IV}]{\text{compound capable of ionizing a hydroxyl moiety}} \quad \underset{\text{(I)}}{R^1(OC_2H_4)_nO^-X^+} \qquad 2$$

R^1 and X^+ are as described above and the mixture of compounds of Formula IV is substantially monodispersed; preferably, at least about 96, 97, 98 or 99 percent of the compounds in the mixture of compounds of Formula IV have the same molecular weight; and, more preferably, the mixture of compounds of Formula IV is a monodispersed mixture.

Various compounds capable of ionizing a hydroxyl moiety on the PEG moiety of the

compound of Formula IV will be understood by those skilled in the art. The compound capable of ionizing a hydroxyl moiety is preferably a strong base. More preferably, the compound capable of ionizing a hydroxyl moiety is selected from the group consisting of sodium hydride, potassium hydride, sodium t-butoxide, potassium t-butoxide, butyl lithium (BuLi), and lithium diisopropylamine. The compoumd capable of ionizing a hydroxyl moiety is more preferably sodium hydride.

The molar ratio of the compound capable of ionizing a hydroxyl moiety on the PEG moiety of the compound of Formula IV to the compound of Formula IV is preferably at least about 1:1, and is more preferably at least about 2:1. By providing an excess of the compound capable of ionizing the hydroxyl moiety, it is assured that substantially all of the compounds of Formula IV are reacted to provide the compounds of Formula I. Thus, separation difficulties, which may occur if both compounds of Formula IV and compounds of Formula I were present in the reaction product mixture, may be avoided.

Reaction 2 is preferably performed between about 0°C and about 40°C, is more preferably performed between about 0°C and about 35°C, and is most preferably performed between about 0°C and room temperature (approximately 25°C).

Reaction 2 may be performed for various periods of time as will be understood by those skilled in the art. Reaction 2 is preferably performed for a period of time between about 0.25, 0.5 or 0.75 hours and about 2, 4 or 8 hours.

Reaction 2 is preferably carried out in an aprotic solvent such as, but not limited to, N,N-dimethylacetamide (DMA), N,N-dimethylformamide (DMF), dimethyl sulfoxide (DMSO), hexamethylphosphoric triamide, tetrahydrofuran (THF), dioxane, diethyl ether, methyl t-butyl ether (MTBE), toluene, benzene, hexane, pentane, N-methylpyrollidinone, dichloromethane, chloroform, tetrahydronaphthalene, decahydronaphthalene, 1,2-dichlorobenzene, 1,3-dimethyl-2-imidazolidinone, or a mixture thereof. More preferably, the solvent is DMF, dichloromethane or toluene.

Compounds of Formula II are preferably prepared as illustrated in reaction 3:

$$\underset{(V)}{R^2(OC_2H_4)_mOH} \quad + \quad CH_3\overset{O}{\underset{O}{\overset{\|}{\underset{\|}{S}}}}Q \quad \longrightarrow \quad \underset{(II)}{R^2(OC_2H_4)_mOMs} \qquad 3$$

R^2 and Ms are as described above and the compound of Formula V is present as a substantially monodispersed mixture of compounds of Formula V; preferably at least about